



IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

19331

MOIPC—84—III-1-93—22-8-15—5,000.

PROCEEDINGS
OF THE
NATIONAL ACADEMY
OF SCIENCES
OF THE
UNITED STATES OF AMERICA

VOLUME 24, 1938



EXECUTIVE COMMITTEE

EDWIN BIDWELL WILSON, *Managing Editor*

RAYMOND PEARL

R. G. HARRISON

PUBLISHED MONTHLY

Publication Office: Mack Printing Company, Easton, Pa.

Editorial Office: Harvard School of Public Health, Boston 17

Home Office of the Academy: Washington, D. C.

CONTENTS

MATHEMATICS

	Page
GROUPS HAVING A MAXIMUM NUMBER SET OF INDEPENDENT GENERATORS.....	
<i>By G. A. Miller</i>	17
SPECIAL HOMOLOGY GROUPS.....	<i>By M. Richardson</i> 21
LOG π AND OTHER BASIC CONSTANTS.....	<i>By H. S. Uhler</i> 23
EINSTEIN SPACES IN A SPACE OF CONSTANT CURVATURE.....	<i>By A. Fialkow</i> 30
CLASSIFICATION OF ELEMENT TRANSFORMATIONS BY MEANS OF ISOGONAL AND EQUI-TANGENTIAL SERIES.....	<i>By E. Kasner and J. de Cicco</i> 34
ABSTRACT SELF-ADJOINT BOUNDARY CONDITIONS.....	<i>By J. W. Calkin</i> 38
GROUPS HAVING A MAXIMUM SET OF INDEPENDENT GENERATORS OF THE SAME ORDER.....	<i>By G. A. Miller</i> 91
GROUPS HAVING A MAXIMUM NUMBER SET OF CONJUGATE INDEPENDENT GENERA- TORS.....	<i>By G. A. Miller</i> 94
THE EXISTENCE OF A MINIMAL SURFACE OF LEAST AREA BOUNDED BY PRESCRIBED JORDAN ARCS AND PRESCRIBED SURFACES.....	<i>By R. Courant</i> 97
THE CONTINUUM PROBLEM.....	<i>By F. Bernstein</i> 101
ON THE EXISTENCE OF A CLOSED CONVEX SURFACE REALIZING A GIVEN RIE- MANNIAN METRIC.....	<i>By H. Lewy</i> 104
DEPENDENT PROBABILITIES AND SPACES (L).....	<i>By G. Birkhoff</i> 154
ON SEMI-GROUPS OF TRANSFORMATIONS IN HILBERT SPACE.....	<i>By E. Hille</i> 159
RESIDUATED LATTICES.....	<i>By M. Ward and R. P. Dilworth</i> 162
THE GENERALIZED CLEBSCH-GORDAN FORMULA.....	<i>By F. D. Murnaghan</i> 182
THE ANALYSIS OF REPRESENTATIONS OF THE REAL ORTHOGONAL GROUP.....	<i>By F. D. Murnaghan</i> 184
NATURAL SYSTEMS.....	<i>By A. L. Foster</i> 185
EXTENSIONS OF MEASURE.....	<i>By H. M. MacNeille</i> 188
REPRESENTATION AND EQUIVALENCE OF FORMS.....	<i>By R. Oldenburger</i> 193
RELATIVE NUMBERS OF OPERATORS AND SUBGROUPS OF A FINITE GROUP.....	<i>By G. A. Miller</i> 199
MINIMUM DEGREE OF SUBSTITUTIONS OF HIGHEST DEGREE IN A GROUP.....	<i>By G. A. Miller</i> 202
ON NORMAL DIVISION ALGEBRAS OF INDEX 5.....	<i>By R. Brauer</i> 243
LARGEST DEGREE OF SUBSTITUTION IN THE GROUPS OF A GIVEN DEGREE.....	<i>By G. A. Miller</i> 293
REMARKS ON RIEMANN'S DOCTORAL DISSERTATION.....	<i>By J. Douglas</i> 297
FUNCTIONAL TOPOLOGY AND ABSTRACT VARIATIONAL THEORY.....	<i>By M. Morse</i> 326
ON CRITERIA CONCERNING SINGULAR INTEGERS IN CYCLOTOMIC FIELDS.....	<i>By H. S. Vandiver</i> 330
GROUPS OF DEGREE n INVOLVING ONLY SUBSTITUTIONS OF LOWER DEGREES.....	<i>By G. A. Miller</i> 333
ON THE DERIVATIVES OF FUNCTIONS ANALYTIC IN THE UNIT CIRCLE.....	<i>By J. L. Walsh and W. Seidel</i> 337
DIFFERENTIAL CALCULUS IN LINEAR TOPOLOGICAL SPACES.....	<i>By A. D. Michal</i> 340
MINIMAL SURFACES OF HIGHER TOPOLOGICAL STRUCTURE.....	<i>By J. Douglas</i> 343
GREEN'S FUNCTION AND THE PROBLEM OF PLATEAU.....	<i>By J. Douglas</i> 353
THE MOST GENERAL FORM OF THE PROBLEM OF PLATEAU.....	<i>By J. Douglas</i> 360
ON LOCALLY CONNECTED SETS AND RETRACTS.....	<i>By S. Lefschetz</i> 392
CONFORMAL GEOMETRY OF HORN ANGLES OF SECOND ORDER.....	<i>By E. Kasner and J. de Cicco</i> 393

THE RELATIVE NUMBERS OF THE SUBGROUPS AND OPERATORS OF CERTAIN GROUPS. . .	<i>By G. A. Miller</i>	473
ON INTERPOLATION AND APPROXIMATION BY FUNCTIONS ANALYTIC AND BOUNDED IN A GIVEN REGION.	<i>By J. L. Walsh</i>	477
A NEW FOUNDATION OF NON-EUCLIDEAN, AFFINE, REAL PROJECTIVE AND EUCLIDEAN GEOMETRY.	<i>By K. Menger</i>	486
A JORDAN SPACE CURVE HAVING THE INFINITE AREA PROPERTY AT EACH OF ITS POINTS.	<i>By J. Douglas</i>	490
REMARKS ON PLATEAU'S PROBLEM AND DOUGLAS' PROBLEM.	<i>By R. Courant</i>	519
THE CONSISTENCY OF THE AXIOM OF CHOICE AND OF THE GENERALIZED CON- TINUUM-HYPOTHESIS.	<i>By K. Gödel</i>	556
THE CHARACTERIZATION OF PSEUDO- $S_{n,r}$ SETS.	<i>By L. M. Blumenthal and G. R. Thurman</i>	557
ON SEMI-GROUPS OF SELFADJOINT TRANSFORMATIONS IN HILBERT SPACE.	<i>By B. de Sz. Nagy</i>	559
GROUPS OF THE ORDER p^m CONTAINING $m - \alpha$ INDEPENDENT GENERATORS.	<i>By G. A. Miller</i>	561

ASTRONOMY

ASYMMETRY OF LIGHT CURVES IN THE GREAT SEQUENCE AS A FUNCTION OF FRE- QUENCY OF PERIOD.	<i>By S. Gaposchkin</i>	1
NOTE ON THE STELLAR DISTRIBUTION IN THE VICINITY OF A SOUTHERN GALACTIC WINDOW.	<i>By B. J. Bok and E. M. Lindsay</i>	4
NOTE ON THE PROBLEM OF THE EXPANDING UNIVERSE.	<i>By H. Shapley</i>	148
A METAGALACTIC DENSITY GRADIENT.	<i>By H. Shapley</i>	282
SOME RECENT OBSERVATIONS OF SUNSPOT SPECTRA.	<i>By H. D. Babcock</i>	525
SECOND NOTE ON A METAGALACTIC DENSITY GRADIENT.	<i>By H. Shapley</i>	527
REFLECTIVITY AND COLOR OF METEORITES.	<i>By F. G. Watson</i>	532

PHYSICS

THE RELATIONS BETWEEN STEFAN'S RADIATION LAW, NERNST'S HEAT THEOREM AND MAXWELL'S FORMULA FOR THE RADIATION PRESSURE.	<i>By E. Guth and A. E. Haas</i>	224
THE LIFT AND DRAG FUNCTIONS FOR AN ELASTIC FLUID IN TWO DIMENSIONAL IRROTATIONAL FLOW.	<i>By H. Bateman</i>	246
THE MECHANISM OF HEARING AS REVEALED THROUGH EXPERIMENT ON THE MASKING EFFECT OF THERMAL NOISE.	<i>By H. Fletcher</i>	265
THE DIMENSIONLESS CONSTANTS OF PHYSICS.	<i>By A. E. Haas</i>	274
RAYLEIGH WAVES.	<i>By H. Bateman</i>	315
COULOMB'S FUNCTION.	<i>By H. Bateman</i>	321
THE MECHANISM OF DELAYED KILLING OF MAIZE SEEDS WITH X-RADIATION. . . .	<i>By L. R. Maxwell</i>	377
THEORY OF TRANSVERSAL CURVES AND THE CONNECTIONS BETWEEN THE CALCULUS OF VARIATIONS AND THE THEORY OF PARTIAL DIFFERENTIAL EQUATIONS.	<i>By M. Herzberger</i>	466

CHEMISTRY

ORGANISMS REQUIRING VITAMIN B ₁	<i>By W. J. Robbins</i>	53
THE SPECIFICITY OF PYRIMIDINE FOR PHYCOMYCES BLAKESLEEANUS.	<i>By W. J. Robbins and F. Kavanagh</i>	141
THE SPECIFICITY OF THIAZOLE FOR PHYCOMYCES BLAKESLEEANUS.	<i>By W. J. Robbins and F. Kavanagh</i>	145

MEASUREMENTS OF RESISTANCE AND CAPACITY OF MONOFILMS OF BARIUM STEARATE	By C. E. Buchwald, D. M. Gallagher, C. P. Haskins, E. M. Thatcher and P. A. Zahl	204
EVIDENCE FOR A SECOND THIAMIN.....	By W. J. Robbins and F. Kavanagh	229

GEOLOGY AND PALEONTOLOGY

✓ CHEMICAL STRATIFICATION AND LAKE MORPHOLOGY.....	By G. E. Hutchinson	63
A TARSIID PRIMATE AND A MIXODECTID FROM THE POWAY EOCENE, CALIFORNIA. . .	By C. Stock	288
PRELIMINARY REPORT ON RECENT GEOLOGICAL AND ARCHAEOLOGICAL DISCOVERIES RELATING TO EARLY MAN IN SOUTHEAST ASIA.....	By H. deTerra	407
A TITANOTHERE FROM THE TYPE SESPE OF CALIFORNIA.....	By C. Stock	507

BOTANY

A CYTOLOGICAL STUDY OF COLCHICINE EFFECTS IN THE INDUCTION OF POLYPLOIDY IN PLANTS.....	By O. J. Figsti	56
• THE ORIGIN OF MAIZE.....	By P. C. Mangelsdorf and R. G. Reeves	303
CONTACT EFFECTS BETWEEN PLANT ROOTS AND SOIL COLLOIDS.....	By H. Jenny and R. Overstreet	384
PARTIAL SELF-INCOMPATIBILITY IN <i>MEDICAGO SATIVA</i>	By R. A. Brink and D. C. Cooper	497

ZOOLOGY AND ANATOMY

THE PRESENT STATE OF OUR KNOWLEDGE CONCERNING THE LIFE CYCLE OF THE FORAMINIFERA.....	By E. H. Myers	10
SEX REACTION TYPES AND THEIR INTERRELATIONS IN <i>PARAMECIUM BURSARIA</i> . I..	By H. S. Jennings	112
SEX REACTION TYPES AND THEIR INTERRELATIONS IN <i>PARAMECIUM BURSARIA</i> . II. CLONES COLLECTED FROM NATURAL HABITATS.....	By H. S. Jennings	117
THE RÔLE OF THE MELANOPHORE-DISPERSING HORMONE OF THE PITUITARY IN THE COLOR CHANGES OF THE CATFISH.....	By C. M. Osborn	121
BIOLOGICAL DIFFERENCES IN THE ACTION OF SYNTHETIC MALE HORMONES ON THE DIFFERENTIATION OF SEX IN THE CHICK EMBRYO.....	By B. H. Willier, M. E. Rawles and F. C. Koch	176
AN ASTACENE-LIKE CAROTENOID FROM A PACIFIC COAST ANEMONE, <i>EPIACTIS PROLIFERA</i>	By D. L. Fox and C. R. Moe	230
FEATHER CHARACTERIZATION AS STUDIED IN HOST-GRAFT COMBINATIONS BETWEEN CHICK EMBRYOS OF DIFFERENT BREEDS.....	By B. H. Willier and M. E. Rawles	446
CONCERNING THE ORIGIN OF THE POLYTENE CHROMOSOMES OF DIPTERA.....	By K. W. Cooper	452
SOME EFFECTS OF LIGHT INTENSITY AND SHADE OF BACKGROUND UPON THE MELANIN CONTENT OF <i>GAMBUSIA</i>	By F. B. Sumner and P. Doudoroff	459
THE EFFECTS OF LIGHT AND DARK BACKGROUNDS UPON THE INCIDENCE OF A SEEMINGLY INFECTIOUS DISEASE IN FISHES.....	By F. B. Sumner and P. Doudoroff	463
MITOTIC BEHAVIOR OF INDUCED CHROMOSOMAL FRAGMENTS LACKING SPINDLE ATTACHMENTS IN THE NEUROBLASTS OF THE GRASSHOPPER. .	By J. G. Carlson	500
AN INTERNAL SECRETION AFFECTING VIABILITY IN CRUSTACEA.....	By F. A. Brown, Jr.	551

GENETICS

CHROMOSOME NUMBERS IN NODULES AND ROOTS OF RED CLOVER, COMMON VETCH AND GARDEN PEA.....	<i>By L. Wipf and D. C. Cooper</i>	87
THE BAR "LOCUS" AND THE v^+ REACTION IN DROSOPHILA MELANOGASTER.....	<i>By A. G. Steinberg and M. Abramowitz</i>	107
INTERSEXUALITY IN DROSOPHILA VIRILUS AND ITS BEARING ON SEX DETERMINATION.....	<i>By G. A. Lebedeff</i>	165
TRANSLOCATION IN RELATION TO MOSAIC FORMATION IN MAIZE... <i>By D. F. Jones</i>		208
THE STRUCTURE OF THE RING-GLAND (CORPUS ALLATUM) IN NORMAL AND LETHAL LARVAE OF DROSOPHILA MELANOGASTER.....	<i>By B. Scharrer and E. Hadorn</i>	236
THE DISTRIBUTION OF GENE FREQUENCIES UNDER IRREVERSIBLE MUTATION....	<i>By S. Wright</i>	253
FACET NUMBER AND THE v^+ HORMONE IN THE BAR EYE OF DROSOPHILA MELANOGASTER.....	<i>By S. Chevais, B. Ephrussi and A. G. Steinberg</i>	365
AN X-RAY INDUCED INTERCALARY DUPLICATION IN DROSOPHILA INVOLVING UNION OF SISTER CHROMATIDS.....	<i>By B. P. Kaufmann and R. C. Bate</i>	368
THE DISTRIBUTION OF GENE FREQUENCIES IN POPULATIONS OF POLYPLOIDS.....	<i>By S. Wright</i>	372
CHROMOSOME PHYLOGENY.....	<i>By C. E. McClung</i>	439
AUTOSOMAL LINKAGE IN MAN—THE RECOMBINATION RATIO BETWEEN CONGENITAL TOOTH DEFICIENCY AND HAIR COLOR.....	<i>By B. Burks</i>	512
THE EFFECTS OF CHEMICALS ON THE LETHAL MUTATION RATE IN DROSOPHILA MELANOGASTER.....	<i>By L. W. Law</i>	546

PHYSIOLOGY AND BIOCHEMISTRY

A SIMPLIFIED METHOD FOR AUXIN EXTRACTION.....	<i>By J. van Overbeek</i>	42
LIGHT INTENSITY AND THE NITROGEN HUNGER PERIOD IN THE MANCHU SOYBEAN	<i>By E. B. Fred, P. W. Wilson and O. Wyss</i>	46
ASCORBIC ACID AND THE GROWTH OF PLANT EMBRYOS.....	<i>By J. Bonner and D. Bonner</i>	70
POTENTIALS IN HALICYSTIS AS AFFECTED BY NON-ELECTROLYTES.....	<i>By W. J. V. Osterhout</i>	75
A COMPARISON OF THE DIFFUSIBLE SUBSTANCES CONCERNED WITH EYE COLOR DEVELOPMENT IN DROSOPHILA, EPHESTIA AND HABROBRACON.....	<i>By G. W. Beadle, R. L. Anderson and J. Maxwell</i>	80
THE EFFECT OF PH ON THE DEVELOPMENT OF ULTRA-CENTRIFUGED FUCUS EGGS	<i>By D. M. Whitaker</i>	85
ON THE DUPLEXITY THEORY OF VISUAL RESPONSE IN VERTEBRATES.....	<i>By W. J. Crozier, E. Wolf and G. Zerrahn-Wolf</i>	125
ON THE LAW FOR MINIMAL DISCRIMINATION OF INTENSITIES. III.....	<i>By W. J. Crozier and A. H. Holway</i>	130
A DETERMINATION OF THE MAGNITUDE OF THE CELL "SENSITIVE VOLUME" ASSOCIATED WITH THE WHITE-EYE MUTATION IN X-RAYED DROSOPHILA. III	<i>By C. P. Haskins and E. V. Enzmann</i>	136
THE SEDIMENTATION CONSTANT OF VISUAL PURPLE.....	<i>By S. Hecht and E. G. Pickels</i>	172
TEMPERATURE AND THE CRITICAL INTENSITY FOR RESPONSE TO VISUAL FLICKER	<i>By W. J. Crozier, E. Wolf and G. Zerrahn-Wolf</i>	216
SPECIFIC CONSTANTS FOR VISUAL EXCITATION. II.....	<i>By W. J. Crozier, E. Wolf and G. Zerrahn-Wolf</i>	221

AUXIN IN ISOLATED ROOTS GROWING IN VITRO.	<i>By J. van Overbeek and J. Bonner</i>	260
CALCULATIONS OF BIOELECTRIC POTENTIALS. III. VARIATION IN PARTITION COEFFICIENTS AND ION MOBILITIES.	<i>By S. E. Hill and W. J. V. Osterhout</i>	312
THE TIME COURSE OF PHOTOSYNTHESIS AS SHOWN BY THE GLASS ELECTRODE, WITH ANOMALIES IN THE ACIDITY CHANGES.	<i>By L. R. Blinks and R. K. Show</i>	413
THE TIME COURSE OF PHOTOSYNTHESIS AS SHOWN BY A RAPID ELECTRODE METHOD FOR OXYGEN.	<i>By L. R. Blinks and R. K. Show</i>	420
REVERSAL OF THE POTASSIUM EFFECT IN NITELLA.	<i>By W. J. V. Osterhout and S. E. Hill</i>	427
SYNTHESIS CARRIED OUT IN VIVO BY ISOLATED PEA ROOTS: I.	<i>By J. Bonner and E. R. Buchman</i>	431
ON THE DUPLEXITY THEORY OF VISUAL RESPONSE IN VERTEBRATES. II.	<i>By W. J. Crozier and E. Wolf</i>	538
SPECIFIC CONSTANTS FOR VISUAL EXCITATION. III. <i>By W. J. Crozier and E. Wolf</i>		542

PATHOLOGY AND BACTERIOLOGY

NOTE ON ESTIMATING BACTERIAL POPULATIONS BY THE DILUTION METHOD.	<i>By R. D. Gordon</i>	212
A SKELETON LIFE TABLE.	<i>By C. R. Doering and A. L. Forbes</i>	400

PSYCHOLOGY AND ANTHROPOLOGY

ON THE RELATIVE CONTRIBUTIONS OF NATURE AND NURTURE TO AVERAGE GROUP DIFFERENCES IN INTELLIGENCE.	<i>By B. S. Burks</i>	276
--	-----------------------	-----

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

January 15, 1938

Number 1

Copyright 1938 by The National Academy of Sciences

ASYMMETRY OF LIGHT CURVES IN THE GREAT SEQUENCE
AS A FUNCTION OF FREQUENCY OF PERIOD

BY SERGEI GAPOSCHKIN

HARVARD COLLEGE OBSERVATORY

Communicated December 9, 1937

The cluster-type variables, the Cepheids, and the long-period variables are generally understood to belong to one large group of stars in which the variations of light, spectrum and radial velocity are considered to have intrinsically the same cause. This group of variable stars may be conveniently called the Great Sequence. The so-called semi-regular variables like RV Tauri are undoubtedly related to the group. All the available light curves of stars of the Great Sequence have recently been investigated by Mrs. Gaposchkin and the writer, and one of the more significant results of the survey is presented in this paper. The complete results will be published elsewhere. There have been previous investigations concerning the relation between the period and the shape of the light curve. Detailed references for long-period variables are given by Ludendorff (*Hand. d. Ap.*, 6, 92, 1928) and Thomas (*Veröff. Ber.-Bab.*, 9, No. 4, 1932), and for Cepheids, by Parenago and Kukarkin (*Zeits. f. Ap.*, 11, 337, 1937).

Differences in shape of light curve suggest classifications. It is characteristic of the Great Sequence that similar light curves are found in all the constituent groups of stars, although there are large differences within any one group. There have been useful attempts, such as those of Ludendorff, Campbell, Thomas, Phillips, Hertzsprung, Parenago and Kukarkin, to classify or describe the variety of light curves, but all of these classifications have been confined to some one single subgroup. We are here concerned with the period-light curve relationships throughout the entire Great Sequence. For this purpose we have selected three parameters to describe the light curve: the ratios a/b , c/d and e/f , where a and b are two areas designated in figure 1a; c and d are two areas designated in figure 1b. The horizontal line has been drawn through the median magnitudes; e is the distance from the "bump" to the following minimum, and f is the distance from the maximum to the minimum. The three ratios furnish a complete description of the light curve, permitting the expression of the

following features: asymmetry with respect to the ordinate (speed of rise and fall); asymmetry with respect to the abscissa (duration of maximum and minimum); existence of a bump or shoulder on the ascending or descending branch. Our ratio a/b is closely correlated with the quantity $(m - M)/P$, which, with some modification, has been extensively used to characterize the light curves of Cepheids, or even long-period variables.

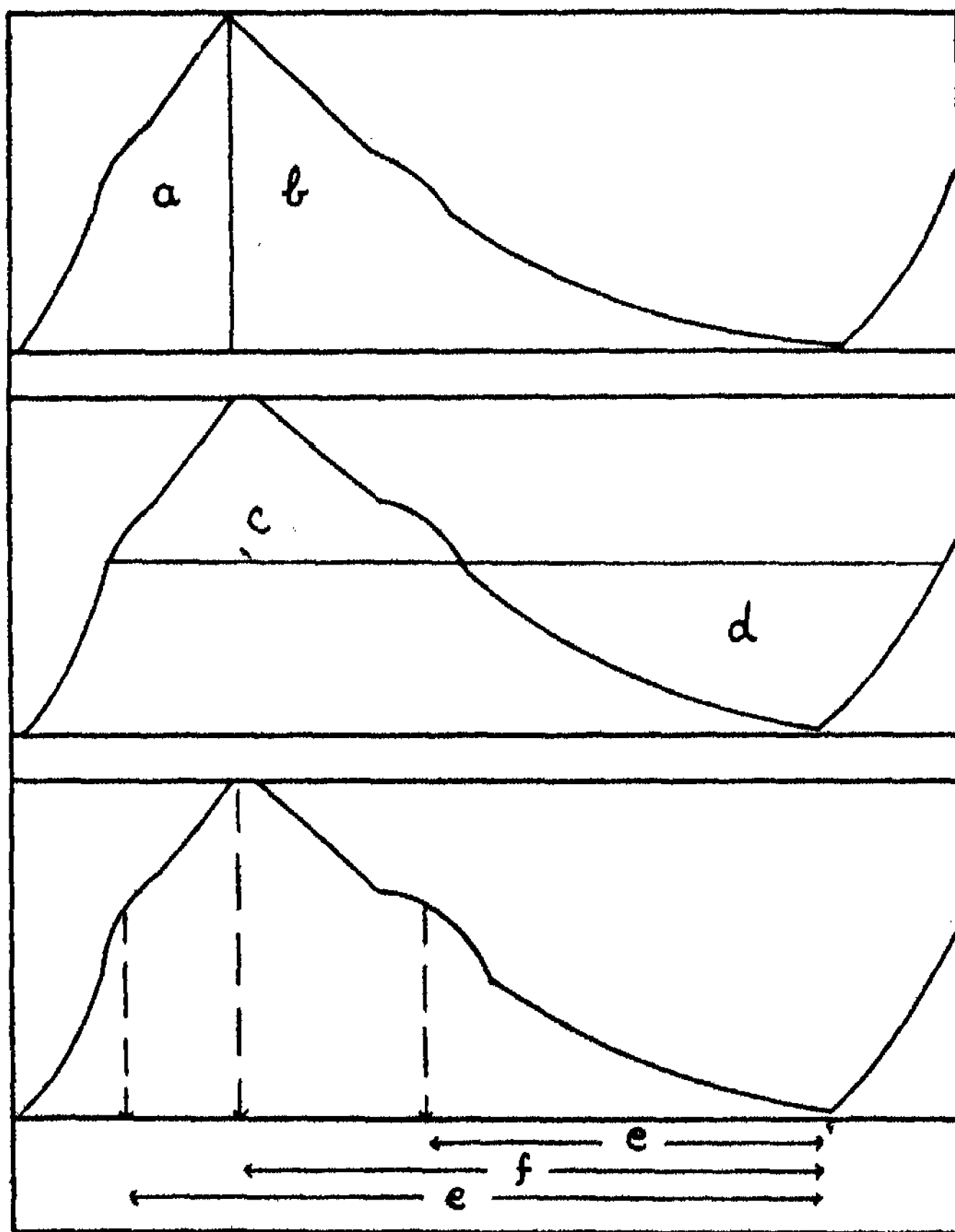


FIGURE 1

Parameters for describing light curve: a/b , c/d and e/f

But $(m - M)/P$ is a less precise and elastic quantity than a/b , especially for light curves with a more or less constant brightness at minimum, for which the position of the actual minimum is indefinite, and the value of $(m - M)/P$ correspondingly doubtful. The ratio a/b is less sensitive to this source of uncertainty.

The general character of the three ratios is illustrated below:

$a/b = 1.0$	Rise and fall equally steep
$a/b < 1.0$	Rise steeper than fall
$a/b > 1.0$	Rise less steep than fall
$c/d = 1.0$	Maximum and minimum equal
$c/d < 1.0$	Maximum shorter (sharper) than minimum
$c/d > 1.0$	Maximum longer (less sharp) than minimum
$e/f < 1.0$	Bump on descending branch
$e/f > 1.0$	Bump on rising branch

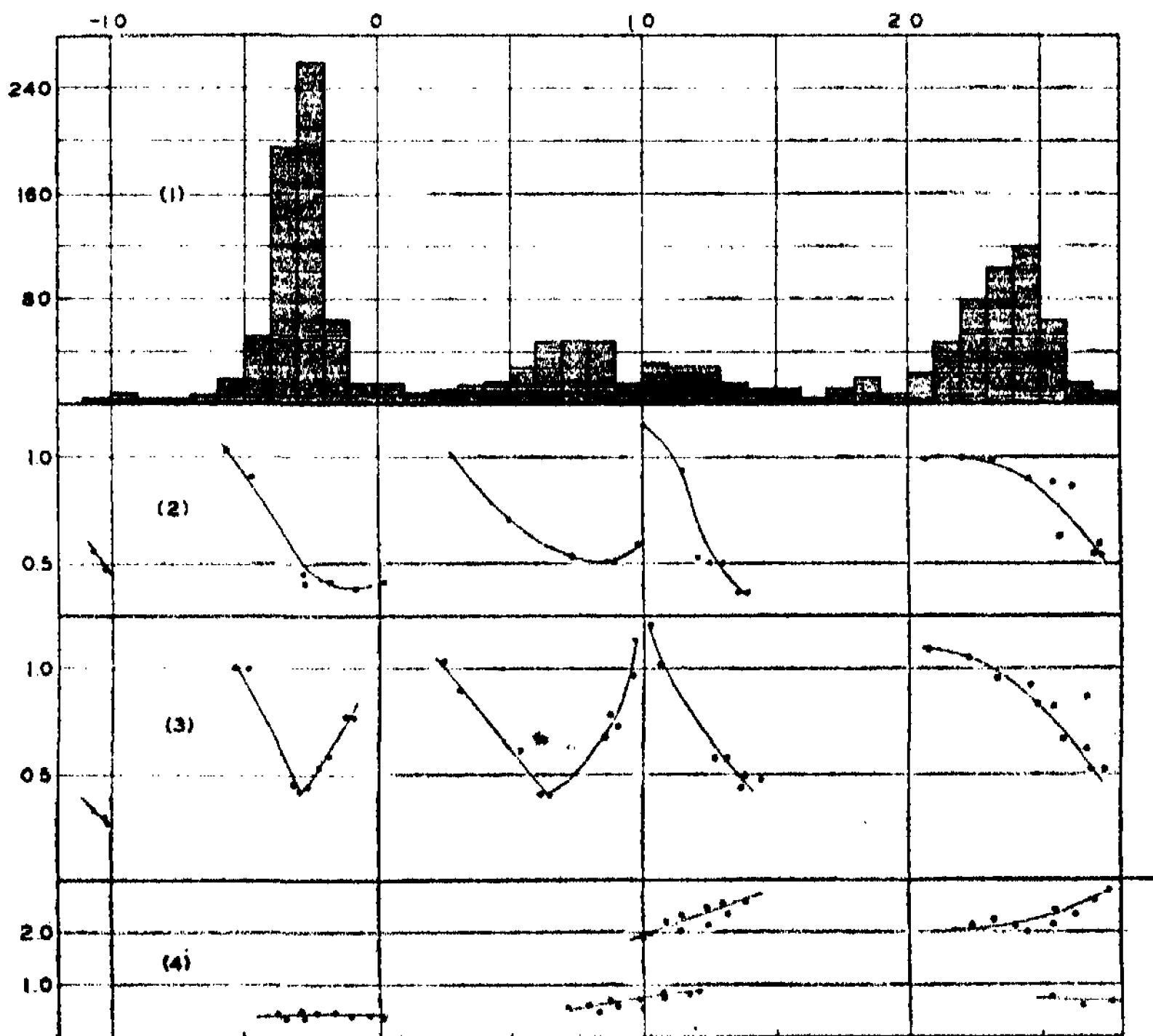


FIGURE 2

Relation of light curve to period in the Great Sequence. Abscissae, logarithms of period; ordinates, (1) the frequency of period, (2) the parameter a/b , (3) the parameter c/d , (4) the parameter e/f .

About a thousand stars belonging to the Great Sequence, including a number of variables in globular clusters, have been investigated. Within each subgroup of the Great Sequence there is large variation in the values of the three ratios. Most of the light curves show some asymmetry. The results shown in figure 2 may be summarized as follows:

(1) There are several maxima and minima of period-frequency. Maxima lie at about $0^d.1$, $0^d.5$, $5^d.0$, $10^d.1$, $85^d.0$, $320^d.0$, the first corresponding to cluster-type variables, and the sixth to the long-period variables, which probably, themselves, constitute a double group. The minima lie at about $0^d.2$, $1^d.6$, $9^d.0$ and $50-60^d.0$, and possibly at about 600^d .

(2) Between any two minima of frequency the light curves run, with increasing period, from symmetry to asymmetry.

(3) The long-period Cepheids, and the long-period variables of longest period, show bumps on the rising branch of the light curve, which are nearest to the minimum for the longest periods. The bumps on the descending branch seem to occur at about the same phase, whatever the period. The cluster-type Cepheids do not show bumps on the rising branch of the light curve.

(4) At the minima of period-frequency the light curves show the greatest irregularities.

It may be remarked that there is no definite indication of a period-spectrum relation within the Great Sequence similar to the relations mentioned above, except perhaps at the minimum near sixty days. It is probable that the groups of stars are not mutually exclusive in period, but overlap slightly; with the two groups of long-period variables the overlap is considerable.

NOTE ON THE STELLAR DISTRIBUTION IN THE VICINITY OF A SOUTHERN GALACTIC WINDOW

BY BART J. BOK AND ERIC M. LINDSAY

HARVARD COLLEGE OBSERVATORY

Communicated December 15, 1937

Harvard and Mount Wilson studies on the distribution of faint external galaxies have led to the discovery of several galactic "windows," regions in comparatively low latitudes which are unusually rich in distant extragalactic nebulae. For most fields within fifteen degrees of the galactic equator, the total absorption of star light in our Milky Way system is so large that very few nebulae are found on a plate. The discovery of a galactic window is by itself excellent evidence for the smallness of the total galactic absorption in that particular direction. The present note deals with the stellar distribution for the most conspicuous southern galactic window, discovered by Shapley, which is located near galactic longitude $\lambda = 300^\circ$, between galactic latitudes $\beta = -10^\circ$ and -16° . An estimate

of the total galactic absorption from the observed frequency of external galaxies gives $\Delta m = 0.4$ for photographic light.

1. *Star Counts*.—Shapley and Miss Boyd¹ have published star counts made by Miss R. Jones from a Bruce plate centered at $\alpha = 16^h 52^m 3$, $\delta = -62^\circ 28'$. The regions at and around the galactic window are, moreover, in the part of the sky covered by Lindsay's unpublished star counts² between $m_{pg} = 10.0$ and 13.5, for the southern galactic belt. Since it will probably be some time before the analysis of Lindsay's complete material will be ready for publication, it appeared of interest to make a preliminary study of the counts for the region in the vicinity of this "window."

Lindsay's star counts suggest the following values of $\log N(m)$ for the center of the Bruce plate counted by Miss Jones:

m_{pg} :	10.0	11.0	12.0	13.0	13.5
$\log N(m)$:	1.00	1.35	1.72	2.00	2.25

The counts by Miss Jones gave the average values:

m_{pg} :	14.0	15.0	16.0	17.0	18.2
$\log N(m)$:	2.47	3.01	3.31	3.84	4.19

$N(m)$ represents in both cases the number of stars of apparent photographic magnitude m or brighter per square degree. Let $A(m)$ represent the corresponding number of apparent magnitude m , defined by the formula $A(m) = dN(m)/dm$. We have assumed in our calculations that the smoothed values of $A(m)$ of table 1 are representative for the southern galactic window.

TABLE 1

STAR COUNTS FOR THE SOUTHERN GALACTIC WINDOW

m_{pg}	$A(m)$	m_{pg}	$A(m)$
10.0	7.7	15.0	965
11.0	17.4	16.0	2430
12.0	41.0	17.0	5780
13.0	103.0	18.0	11900
14.0	330.0		

Lindsay's star counts for neighboring fields show that the star counts to $m = 13.5$ do not indicate the presence of an excess of stars for the region of the galactic window. The curves for the variation of $\log N(m)$ with the galactic longitude for various zones of galactic latitude show no conspicuous variations between $\lambda = 280^\circ$ and $\lambda = 320^\circ$ for the zones $\beta = -8^\circ$ to -12° and $\beta = -13^\circ$ to -17° . The star counts prove that between $m = 10.0$ and 13.5 the values of $\log N(m)$ in the galactic window do not differ by more than ± 0.10 from those for the regions outside the window at the same latitude. Lindsay's counts were made from plates

taken for the purpose with the 8-inch Bache refractor, and great care was taken to obtain counts on a homogeneous photometric system. We feel justified therefore, to conclude that *the numbers of stars brighter than $m_{pl} = 13.5$ for the region of the galactic window do not differ significantly from those of neighboring fields at the same galactic latitudes.*

Comparable data are not yet available for the fainter magnitudes. A comparison between Miss Jones's counts and average numbers of stars, published by van Rhijn,³ shows the region of the galactic window to have fewer stars than nearby comparison regions at the same latitude. The photometric standards for faint southern stars are very uncertain, but the comparison renders it extremely unlikely that a significant excess in the window is present at $m = 18.0$.

The absence of an appreciable excess of stars brighter than the fifteenth magnitude is confirmed by an inspection of plates of one to two hours' exposure taken with short focus cameras whose apertures vary between one and three inches. The stellar distribution for the region between $\lambda = 280^\circ$ and 320° , and $\beta = -10^\circ$ to -20° is apparently quite regular.

2. *Absorption Problems.*—It is of interest to determine the maximum permissible value of the total galactic absorption for photographic light for the regions ten to fifteen degrees on either side of the galactic window but at the same galactic latitude. Since there is no evidence to the contrary, we may assume that the same distribution of star density will hold for the direction of the galactic window as for the comparison regions on either side. The run of the space densities for the direction of the galactic window may be determined from the star counts of table 1. The total absorption for photographic light for the direction of the galactic window is of the order of $0^m.4$. It makes little difference for the analysis how this small amount of absorption is distributed along the line of sight; we have assumed a uniform absorption of $0^m.1$ per 100 parsecs over the first 400 parsecs. The run of the space densities for the direction of the galactic window is shown in table 2.

TABLE 2

DISTRIBUTION OF SPACE DENSITIES FOR GALACTIC WINDOW

r	s	$D(r)$	r	s	$D(r)$
100	20	1.00	1600	320	0.20
160	32	0.85	2500	500	0.15
250	50	0.70	4000	800	0.11
400	80	0.55	6300	1260	0.08
630	126	0.40	10000	2000	0.05
1000	200	0.30			

In table 2, r is the distance in the direction of the galactic window, and $D(r)$ the corresponding space density. The latitude of the region for

which the star counts are available is $\beta = -13^\circ$ on the Harvard pole, and $\beta = -10^\circ$ on van Rhijn's pole; the value $\beta = -11.5^\circ$ for which $\sin \beta = 0.20$ has been assumed for the average latitude of the counted region. The relation between z , the distance above the galactic plane, and the corresponding value of r is then simply: $z = r/5$. The change of luminosity function with increasing distance from the galactic plane has been taken into account;⁴ the tabulated star densities refer to the stars with $M = 0$ to $+5$.

It is now a simple matter to predict the run of the $\log A(m)$ values for one of the comparison regions at $\beta = -11.5^\circ$ in which very few faint galaxies are observed, on various assumptions as to the total absorption for photographic light in the comparison regions. The computations have been carried through for three values of the total absorption, $\Delta m = 1^m.0$, $\Delta m = 2^m.0$ and $\Delta m = 3^m.0$. For lack of better information we shall assume in each case that the absorption is uniformly distributed over the interval $0 < r < 1500$ parsecs and that no absorption occurs beyond $r = 1500$ parsecs. The exact way in which the absorption is distributed along the line of sight is immaterial for the following arguments.

The predicted values of $\log A(m)$ will be found in table 3 and again in graphical form in the accompanying diagram; the $\log A(m)$'s for the region of the galactic window have been given for comparison.

TABLE 3

PREDICTED VALUES OF $\log A(m)$ FOR THREE OBSCURED COMPARISON REGIONS

m	GALAC. WINDOW (OBSERVED)	$\Delta m = 1^m.00$	$\Delta m = 2^m.00$ (PREDICTED)	$\Delta m = 3^m.00$
10.0	0.89	0.80	0.73	0.65
11.0	1.24	1.11	1.00	0.89
12.0	1.61	1.50	1.32	1.19
13.0	2.01	1.90	1.65	1.45
14.0	2.52	2.29	1.98	1.76
15.0	2.98	2.69	2.34	2.04
16.0	3.39	3.11	2.72	2.37
17.0	3.76	3.49	3.11	2.67
18.0	4.07	3.87	3.50	3.12

Lindsay's counts for $m_{pg} = 10.0$ to 13.5 showed no indications of the presence of an excess of stars in the galactic window. It was pointed out that an inequality of the order of ± 0.10 in the $\log N(m)$'s—and presumably a similar variation in the $\log A(m)$'s—might have escaped detection. A comparison between the $\log A(m)$'s in the second column with those in the fourth and fifth columns shows that the cases $\Delta m = 2^m.00$ and $\Delta m = 3^m.00$ are definitely out of the picture. The somewhat less accurate star counts for $m_{pg} = 14$ to 18 fully confirm this conclusion: either the photographic magnitudes used by Miss Jones or those used by

van Rhijn would have to be in error by more than a magnitude at $m_{pg} = 18.0$, if the total photographic absorption in the comparison regions were as large as $\Delta m = 2^m.00$.

The available evidence on stellar distribution shows that *the total photographic absorption for the regions at $\beta = -10^\circ$ to -15° between $\lambda = 280^\circ$ and $\lambda = 320^\circ$ does not exceed $\Delta m = 1^m.50$, and that a more probable value is $\Delta m \leq 1^m.00$. A total absorption $\Delta m = 1^m.00$ for a region at $\beta = -11.5$ implies that the half-value for what has commonly been designated as the*

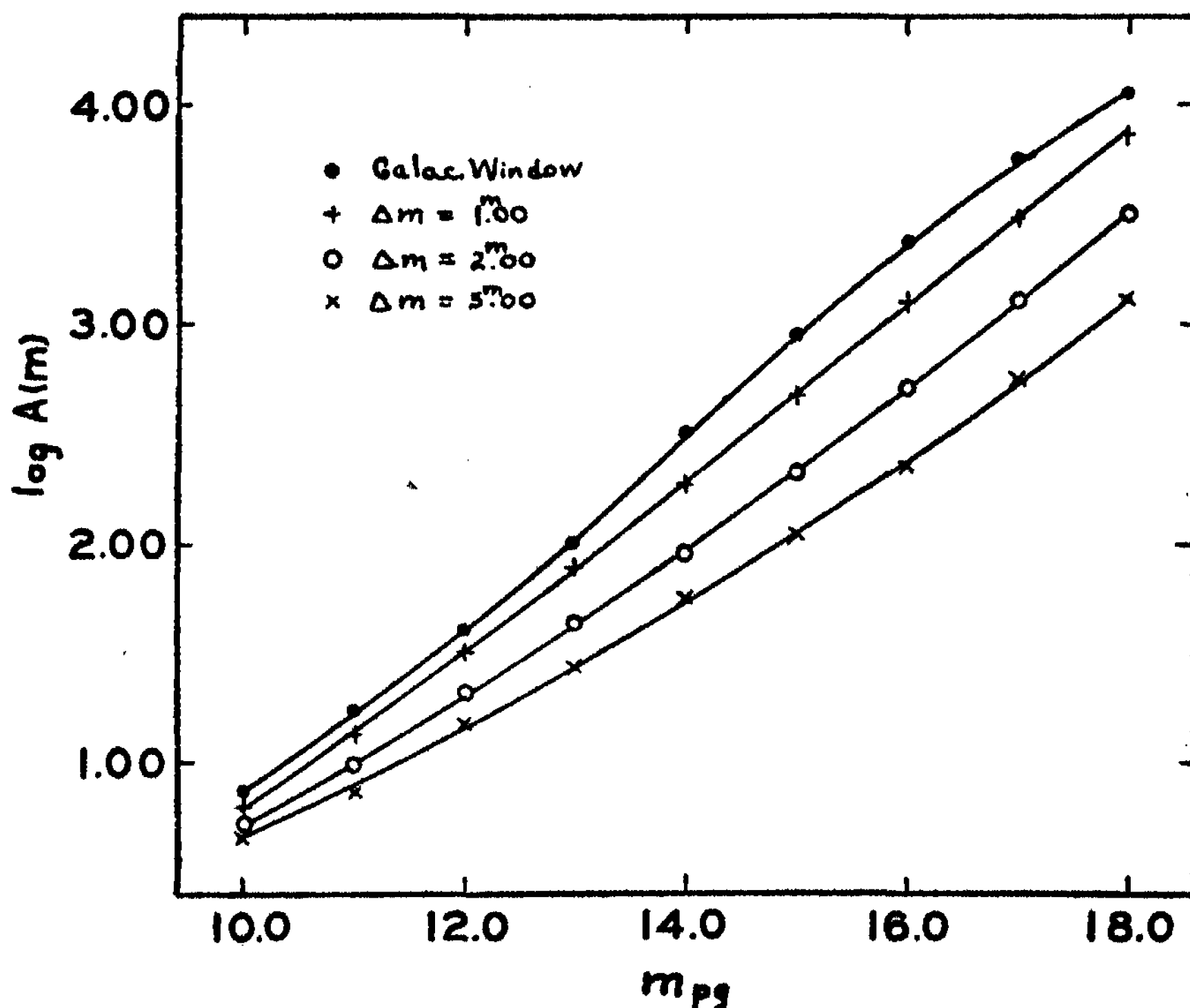


FIGURE 1

"optical thickness" of the galactic absorbing layer is of the order of $0^m.20$ for this particular part of the sky. It is doubtful whether the term "optical thickness" means very much for an absorbing layer that is apparently far from homogeneous, but the value $0^m.20$ may have some significance when applied as an average for a region covering at least 40° in galactic longitude, for which no conspicuous variations in the stellar distribution have been observed.

No certain conclusions as to the amount of the average coefficient of absorption per unit distance in the galactic plane can be derived from ob-

servations at $\beta = -11.5$. The galactic absorption is probably largely confined to a layer with a half-thickness of 200 to 400 parsecs. Our results render it unlikely that the average coefficient of absorption for photographic light near the galactic plane will exceed 1^m0 per kiloparsec.

3. *Suggestions for Future Work.*—It is important that the suggested value $\Delta m \leq 1^m00$ for the entire region $\beta = -10^\circ$ to -15° and $\lambda = 280^\circ$ to 320° be tested. The stellar distribution for this part of the Milky Way seems to be regular. The location of the region under discussion between the center of the greater galactic system and the possible center of a local system makes this part of the sky one of the most interesting for galactic research. Fortunately there is here no evidence for an extended dark nebula similar to the Ophiuchus nebula, which distorts the true features of galactic structure between longitudes $\lambda = 300^\circ$ and 340° north of the galactic plane.

If future studies on the distribution of faint external galaxies give a value $\Delta m > 1^m50$ for the regions on either side of the galactic window, it will probably be necessary to explain the abundance of galaxies in the window by a metagalactic cloud of distant nebulae. It appears most unlikely that the star counts on which our discussion is based would be so much in error as to alter the broad conclusions of the present note. The only remaining alternative would be to account for the galactic absorption by a peripheral absorbing medium which would only become effective at a distance of at least 1000 parsecs below the galactic plane; such a hypothesis cannot be accepted unless supported by strong independent evidence.

The absorption and structural properties of the region as a whole will become better known if studies on the distribution of faint external galaxies go hand in hand with studies on the distribution of colors and variable stars, spectral surveys and star counts to faint magnitude limits on a definite photometric system. For several years our ignorance concerning galactic absorption has appeared to be the chief obstacle in the way of effective analysis of galactic structure; this obstacle can now be removed. Our knowledge of galactic structure can be further advanced if detailed studies of the distribution of stars and nebulae are made for more or less obscured fields near galactic windows as well as for the windows themselves.

¹ *Harvard Annals*, 105, 252 (1937).

² Bok, *The Distribution of the Stars in Space*, 59 (1937).

³ *Groningen Publications* 43 (1929).

⁴ *Zeit. f. Ap.*, 10, 161 (1935).

THE PRESENT STATE OF OUR KNOWLEDGE CONCERNING THE LIFE CYCLE OF THE FORAMINIFERA

BY EARL H. MYERS

COMPTON JUNIOR COLLEGE, COMPTON, CALIF.

Communicated November 20, 1937

The recent extensive use of Foraminifera in solving problems of stratigraphic geology and the recognition of their value as guide fossils in petroleum geology has greatly stimulated interest in the biological problems of these marine rhizopods, consequently many investigators are now contributing to our knowledge of various features of these organisms. Recent contributions include studies of the cytological behavior of the nuclei in reproduction, the rate of reproduction, the life span, those factors which limit their geographic and bathymetric distribution in the sea and the interpretation of the stratigraphic distribution of geological populations on the floor of present oceans from core-samples of sediments.

The conclusions set forth in this paper are the result of one phase of the coöperative study of the Foraminifera planned by Dr. T. Wayland Vaughan and continued under the direction of Dr. H. U. Sverdrup at the Scripps Institution of Oceanography at La Jolla.

In the investigation of the life cycle of the Foraminifera Lister (1895) first explained that the occurrence of two distinct types of individuals within a recognized species was due to an alternation of generations. He described in *Polystomella crista* the asexual origin of megalospheric young from individuals of the larger microspheric generation and presumed that flagellated organisms observed in megalospheric tests were gametes, i.e., zoospores. He further presumed that following the union of these gametes in fertilization, the resulting amoebula gave rise to the asexual or microspheric generation. Many attempts have been made to substantiate these views, but much of the evidence thus far presented has been fragmentary and controversial.

In 1935 the life cycle of the Foraminifera was for the first time brought into agreement with modern biological concepts as they apply to Protozoan cytology. In the life cycle of *Patellina corrugata* (Myers '35) it was abundantly demonstrated that all nuclei were the result of mitotic division, that the nuclei were confined within a nuclear membrane at all times, and that the haploid gametes were amoebulae and not flagellated. The events in this life cycle have been recorded in a motion picture produced under the direction of Dr. C. A. Kofoed, in which forty-one days of reproductive and developmental activity were photographed. The conclusions resulting from this work were further borne out in the life cycle of *Spirillina vivipara* (Myers '36).

There is little doubt that these more recent life cycles have proved confusing, in that they seem to bear slight resemblance to the life cycle of *Polystomella crista* which has been generally accepted as typical of the Foraminifera, but basically the two are similar. Dimorphism is common to both. This dimorphism is the result of an alternation of generations. In both, the test of the asexually produced megalospheric generation is smaller and contains but a single nucleus, while that of the larger sexually produced microspheric generation contains many nuclei. That there is a difference in the nature of the gametes and a presumptive difference in the behavior of the nuclei in reproduction is incidental to this basic similarity.

Evidence further supporting the idea that there is a relationship between the life cycle of *P. corrugata* and *S. vivipara* and those previously proposed was studied this summer for the fifth successive season. In this investigation it was found by prolonged observation of living organisms *in situ* and permanent cytological preparations that the sequence of events in the life cycle of certain species of *Discorbis* and *Bulimina* resemble not only that of *Polystomella crista* proposed by Lister (1895) and Schaudinn (1903) and substantiated in part by Winter in *Peneroplis pertusus* (1907), but also bears a marked resemblance to that of *Spirillina vivipara* and *Patellina corrugata*.

In the life cycle of *P. corrugata* and *S. vivipara* gametogenesis, fertilization and the development of the juvenile stage of the asexual generation takes place within a cyst formed about two or more megalospheric individuals associated for the purpose of sexual reproduction. A similar association of individuals, prior to sexual reproduction, occurs in the species of *Discorbis* and *Bulimina* referred to above, but with these modifications. In these species the tests of megalospheric organisms so associated are arranged with the ventral surfaces opposed, while in the former the several tests remain in contact with the substratum and are enclosed within a protective cyst, the gametes are flagellated and not amoeboid, and the juvenile stage of the microspheric generation develops within the common space formed by the dissolution of a portion of the ventral wall and the septa separating the later formed chambers of the associated tests rather than within an enveloping cyst. In *Discorbis opercularis* d'Orbigny and *Discorbis patelliformis* Brady, the diameter of the initial chamber of the sexually produced microspheric generation is not limited by the size of the zygotic amoebula which resulted from the union of two gametes in fertilization, since considerable growth takes place in the zygotic amoebula following fertilization and before the secretion of the microspheric proluculum, the gametes which failed in fertilization serving as food. In *Patellina* and *Spirillina* similar growth activities in the zygotic amoebula prior to test secretion was also observed. Again we find no evidence in support of the

idea that division of a nucleus of a foraminifer ever results in the production of more than two daughter nuclei.

The dimorphic characters of the respective stages in the life cycle of *Polystomella crista* and *Discorbis opercularis* are in close agreement in regard to the relative diameter of the initial chamber of microspheric and megalospheric tests, the number and arrangement of the chambers and the number of nuclei present in each. These characteristics, together with the flagellate nature of the gametes, make it evident that the cytological behavior of the nuclei in reproduction in *Polystomella* and similar dimorphic species should resemble that observed in the species under discussion.

In *D. opercularis* and *D. patelliformis* we have demonstrated for the first time a genetic continuity in the origin of gametic nuclei from the original megalospheric nucleus by an orderly series of nuclear divisions, in a species in which the gametes are flagellated. Further, we have determined from serial sections of more than five hundred pairs of individuals associated for the purpose of sexual reproduction, that fertilization and the development of the zygotic amoebulae into two and three chambered multinucleate microspheric juveniles occur within the brood chamber formed by an associated group of megalospheric tests, thus establishing on demonstrable evidence, both the origin and fate of flagellated gametes in a foraminifer. The association of megalospheric individuals for the purpose of sexual reproduction and the escape of the sexually produced microspheric young from these associated groups of tests have been observed scores of times in cultures maintained in the laboratory.

The occurrence of free flagellated gametes or zoospores derived from megalospheric *Polystomella crista* has been discussed. Although these observations are in need of verification, we have evidence which tends to support the life cycle proposed in this species by Lister. Since the gametes of *Polystomella* are in all probability free and pelagic, a minimum of opportunity for fertilization is afforded, compared with a state in which the gametes derived from several individuals are confined at all times within a limited area. This difference might explain the occurrence of only one microspheric to more than thirty megalospheric tests in *Polystomella crista* while in *Discorbis opercularis*, where fertilization and the development of the microspheric generation take place within a brood chamber formed by a group of associated tests, the ratio is less than one to two.

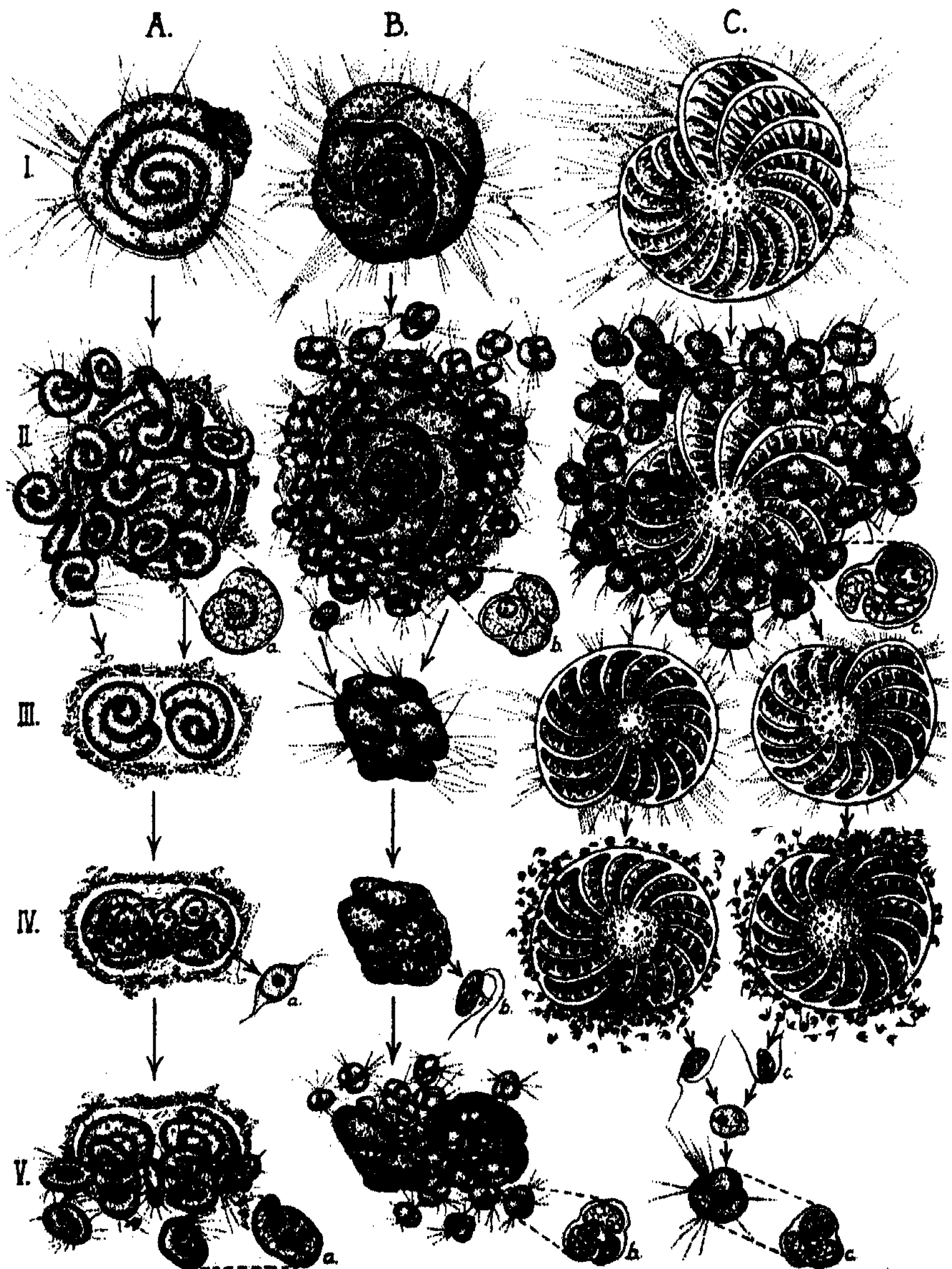
If the gametes, i.e., zoospores, are free and pelagic in those species of foraminifera in which an association of individuals prior to sexual reproduction does not occur, dissemination and distribution may be largely dependent upon this pelagic stage (Vaughan '33).

In *Spirillina vivipara* (Myers '36) there is a reversal in the direction of rotation of the spiral chamber of the tests of the two generations. That a similar condition exists in other species has been reported by Føyn (1936).

This discovery affords an additional characteristic by which one may at times distinguish megalospheric and microspheric tests. Thus we see that in a species such as *Discorbis opercularis* there are several characteristics by which representatives of the respective generations may be identified. In this species the microspheric test contains about forty nuclei, the chambers are usually arranged clockwise in a spire around the initial chamber or proloculum, and this initial chamber is small, while in the megalospheric test only one nucleus is present, the direction of rotation of the chambers is counter-clockwise, and the proloculum is larger.

Three distinct types of tests have been described in certain recognized species of foraminifera. These observations led Hofker (1930) to propose that more than one asexual generation may intervene between two successive sexual generations. Although Hofker's work has met with much criticism, the plan of the life cycle proposed by him is common to other groups of Protozoa and there is no reason why such a life cycle could not occur in the Foraminifera. The existence of such a life cycle is further suggested by the occurrence of many successive asexual generations in isolation cultures of species showing several distinct types of asymmetry. Asexual reproduction of these monoculate and therefore megalospheric foraminifera has been proven cytologically. Since sexual reproduction is known in other species of the same genus, it would be interesting to determine what would happen if isolation cultures of megalospheric organisms of one of these recognized dimorphic species were to be maintained for an appreciable period after they would have reproduced sexually had the opportunity presented itself.

Polymorphism of a different type has been studied by us in *Tretomphalus bulloides*. The life cycle of this interesting species includes an alternation of generations similar to that described in *Discorbis opercularis* but with this difference. When the asexually produced mononucleate megalospheric bottom dwelling stage reaches maturity, a float chamber containing a spherical gas-filled float is added to the ventral side of the test. If this test is ruptured immediately before it assumes the pelagic phase it is found to contain many thousands of biflagellated gametes and a small amount of residual protoplasm which continues to give rise to pseudopodia after the organism has arrived at the surface. This transformation from a bottom-dwelling to a pelagic stage requires about eighteen hours and its developmental stages have been recorded in series of photographs of living organisms. This pelagic stage is of short duration for all of the gametes are discharged within six hours from the time the test arrives at the surface. When two of these pelagic individuals approach one another they are drawn together by anastomosing pseudopodia so that the large pores in the ventral sides of the float chambers are brought into juxtaposition and the



gametes immediately begin to pour out in a cloud about the two tests, thus providing an opportunity for cross fertilization. This pelagic stage of *Tretomphalus* is perhaps one of the most remarkable adaptations of a species to assure the wide dissemination of progeny that occurs within the animal kingdom. The bottom-dwelling or megalospheric stage which gives rise to the pelagic *Tretomphalus* bears a marked resemblance to

EXPLANATION OF PLATE

The life cycle of many foraminifera consists of an orderly succession of sexual and asexual phases. This alternation of generations results in test dimorphism. Five stages are diagrammatically shown on Plate I for three species. (A) *Spirillina vivipara*, (B) *Discorbis patelliformis*, and (C) *Polystomella crispa*.

Asexual Generation

Stage I. In *D. patelliformis* and *P. crispa* as in many foraminifera the initial chamber or proloculum of the sexually produced multinucleate agamont is smaller than that of the asexually produced mononucleate gamont and are known as microspheric and megalospheric respectively, while in *S. vivipara*, a more primitive species, there is little difference in the diameter of the proloculum of the two generations. In all species studied, the agamont test is the larger.

Stage II. Following an orderly series of nuclear divisions multiple fission results in as many mononucleate agamonts as there were nuclei produced. In *D. patelliformis* multiple fission and test secretion takes place within the parent test while in *S. vivipara* and *P. crispa* these activities are preceded by the escape of the protoplasmic content from the test. Stage II. (a, b and c) Enlargement of sexual gamont showing the nucleus.

Sexual Generation

Stage III. In *S. vivipara*, sexual reproduction is preceded by the association of gamonts within a cyst composed of substrate debris and animal cement. A somewhat similar association takes place in *D. patelliformis* where no cyst is formed, while in *P. crispa* it is presumed that a close association between gamonts does not occur, the gametes, or zoospores being free and pelagic.

Stage IV. The gametes of *S. vivipara* are amoeboid while those of *D. patelliformis* and *P. crispa* are flagellated. Gametogenesis includes an orderly series of nuclear divisions of the equational type followed by a reduction division. Fertilization takes place between gametes derived from different gamonts. In *S. vivipara* the agamonts develop within a cyst, in *D. patelliformis* within the space formed by the dissolution of septa between the chambers, while in *P. crispa* the gametes are pelagic, fertilization depending upon the chance meeting of gametes. Stage IV. (a, b and c) Enlargement of gametes.

Stage V. Juvenile agamonts are multinucleate as a result of an orderly series of nuclear divisions immediately following fertilization and before the secretion of the test. Agamonts of *S. vivipara* contain but four nuclei while those of *D. patelliformis* and *P. crispa* each have about forty nuclei. In *S. vivipara* the sexually produced young develop within a cyst, those of *D. patelliformis* within the space resulting from the dissolution of the septa between chambers and the ventral surface of associated tests, while those of *P. crispa* develop from the free zygotic amoebula. Stage V. (a, b and c) Enlargement of asexual agamont showing nuclei.

Discorbis globularis. The microspheric stage is larger, more depressed in appearance, and the initial chamber does not approach the diameter of the smallest megalospheric proloculum observed.

Although our observations on *Tretomphalus* extends over a period of five years, some cultures persisting for as long as three years, we have been unable to demonstrate the fate of the gametes beyond their union in fertilization, however, the genetic origin of the gametes has been determined from cytological preparations.

The cytological problem in the study of life cycles of foraminifera is complicated by the presence of many types of cytoplasmic inclusions. Residual chromatin derived from the nuclei of food organisms and other metabolic products which react with stains that are partially specific for chromatin frequently produce confusing results. Symbiotic algae and other organisms contained within the cytoplasm, or occasionally filling all of the chambers of a test, are at times even more confusing. Various flagellates and amoebae, which we have observed, seem in some instances to be limited to certain species. We have also found a nematode worm which during the colder months of the year has an incidence of more than five per cent in *Rotalia turbinata* Cushman and Valentine. Small pieces of flesh of organisms allowed to decompose in sea water provide a substratum for many types of organisms, especially flagellates. Dead foraminifera in cultures frequently present a similar condition and these flagellates might easily be mistaken for gametes.

There is a decided need for further study of life cycles in larger foraminifera and virtually nothing is known concerning the life cycle of those pelagic groups which are common to most marine sediments, and which frequently constitute the major portion of these deposits. It is to be expected that the revived interest in the biological studies in this group will soon result in the solution of these and other problems, but it will require much time and diligence on the part of the investigator to eliminate those factors which have caused many to question the value of much of the evidence presented in the past.

Contributions from Scripps Institution of Oceanography. New Series, Number 8. Føyn, Bjørn, "Foraminiferenstudien. I. Der Lebenszyklus von *Discorbina vilardeboana* d'Orbigny," *Bergens Museums Arbok, Naturvidenskapelig rekke* Nr. 2 (1936).

Hofker, J., "Foraminifera of the Siboga Expedition, Part 2," *Siboga Expeditie 4a* Leiden (1930).

Lister, J. Jackson, "Contributions to the Life-History of the Foraminifera," *Phil. Trans. Roy. Soc.*, **B186**, 401-453, pls. 6-9 (1895).

Myers, Earl H., "The Life-History of *Patellina corrugata* a Foraminifer," *Bull. Scripps Inst. of Oceanography Calif. Tech. Ser. 3*, No. 15, Univ. of Calif. Press, Berkeley, California (1935).

"The Life Cycle of *Spirillina vivipara*, with Notes on Morphogenesis, Systematics and Distribution in the Foraminifera," *Jour. Roy. Microscopical Society*, **56**, pp. 120-146 (1936).

Schaudinn, F., "Untersuchungen über die Fortpflanzung einiger Rhizopoden," *Arb. Kais. Gesundheitsamte*, 3. Bd., 19, Heft 3, 547-576 (1903).

Vaughan, T. Wayland, "The Biogeographic Relations of the Orbitoid Foraminifera," *Proc. Nat. Acad. Sci.*, 19, No. 10, 922-938 (1933).

Winter, F. W., "Zur Kenntniss der Thalamaporen. I. Untersuchungen über *Peneroplis pertusus*," *Arch. Protist.*, 10, 83 (1907).

GROUPS HAVING A MAXIMUM NUMBER SET OF INDEPENDENT GENERATORS

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated November 25, 1937

A group G is said to have a maximum number set of independent generators whenever it has at least one set of independent generators which involves as many operators as there are prime factors in the order of the group. Each of the operators of such a set is obviously of prime order and if two such operators are of different orders they generate a group whose order is the product of these orders. When two such operators are of the same order they generate a group whose order is either the square of this common order or the product of this common order and a larger prime number which is congruent to unity with respect to this common order. All the operators of the latter group except those in the invariant cyclic subgroup whose order is this larger prime number have the same order.

Every subset composed of k of the operators of a maximum number set of independent generators of such a group generates a subgroup of G whose order is the product of exactly k prime numbers and hence has itself a maximum number set of independent generators. Every operator in such a subset therefore generates a cyclic subgroup which is invariant under all the operators of smaller order contained in this subset. In particular, every operator of highest order in the subset generates an invariant cyclic subgroup under the subset whenever it contains only one operator of this order. When no two of the operators of the subset have the same order the order of the subgroup generated by it is the product of the orders of the operators of the subset but when at least two of these operators have the same order the order of the subgroup generated by it may exceed the product of the orders of these operators.

There is at least one subset composed of operators of smallest order contained in the given maximum number set of independent generators of G which generates a Sylow subgroup of G whose order is a power of the small-

est prime number which divides the order of G since the order of the subgroup generated by a subset cannot be less than the product of the orders of the operators contained in the subset. If a Sylow subgroup of G whose order is a power of the smallest prime number p which divides the order of G is of order p^k and the given maximum number set of independent generators would not contain k operators of order p which generate such a Sylow subgroup then every k of its operators would generate a group whose order would exceed p^k . By adjoining successively an operator from the given maximum number set there would result each time a group whose order is equal to the order of the preceding group multiplied by a larger prime number than p but which divides the order of G . This is obviously impossible since the number of such equal prime numbers could not exceed the index of a power of a prime which is equal to the order of a Sylow subgroup.

From the preceding paragraph it results that a maximum number set of independent generators of G contains k relatively commutative operators which generate a Sylow subgroup of G whose order is a power of the smallest prime number which divides the order of G . It does not necessarily contain any operator which appears in any other Sylow subgroup of G even when the order of G is divisible by various different prime numbers. This is illustrated by the fact that the direct product of an arbitrary number of dihedral groups whose separate orders are the doubles of arbitrary odd prime numbers has a maximum number set of independent generators composed of operators of order 2 while its Sylow subgroup whose order is a power of 2 is of order 2^k , where k is the number of these odd primes, which may be either equal or unequal. It is obvious that in this case a maximum number set of independent generators can also be so selected that each of its operators appears in some Sylow subgroup of G . We proceed to prove that such a selection of the operators of a maximum number set of independent generators is always possible whenever G has a maximum number set of independent generators.

For the sake of simplicity we shall first restrict our attention to the case when the order of G is divisible by only two different prime numbers. When the given maximum number set of its independent generators involves exactly as many operators of lowest order as the index of the highest power of the smaller prime which divides the order of G then it results directly from the preceding proof that G contains two abelian Sylow subgroups such that each is of a type which is of the form 1^k and that each operator in a set of generators of the Sylow subgroup whose order is a power of the larger prime generates a cyclic subgroup which is invariant under G . If the given maximum number set of independent generators of G involves more operators of lowest order than the number of the independent generators of a Sylow subgroup of G whose order is a power of the smallest prime

which divides the order of G we adjoin to the generators of such a Sylow subgroup which appear in the given set a generator of the same order which also appears therein but not in the given Sylow subgroup.

These generators give rise to a group whose order is a prime number times the order of this Sylow subgroup. This group has for a maximum number set of independent generators the given generators of this Sylow subgroup and an operator whose order is this prime number. The cyclic group generated by this operator is invariant under this extended group since it is invariant under the given Sylow subgroup of G . This invariance results from the fact that its two given generators and any other generator of the given Sylow subgroup must generate a group having a maximum number set of three independent generators. If the given maximum number set of independent generators of G involves an operator of the lower order which is not contained in the given extended group we proceed similarly with a second extension. The order of the group resulting from this second extension is equal to the order of the given Sylow subgroup of G multiplied by the square of the largest prime which divides the order of G . This group contains a Sylow subgroup whose order is this square and whose two generators separately generate groups which are invariant under the given Sylow subgroup of G .

The given process can be continued until all the operators of lowest order in the given maximum number set of independent generators of G have been exhausted and as the operators of higher order contained in this set (if any) can be adjoined to those thus obtained so as to obtain an abelian Sylow subgroup whose order is a power of the larger of the prime numbers which divide the order of G . This abelian group is clearly of type which is of the form 1^k and the group generated by each of a set of generating operators is invariant under G . Operators composed of a set of independent generators of each of its two Sylow subgroups clearly constitute a maximum number set of independent generators of G . It should be noted that when operators in the given maximum number set of independent generators are replaced by operators of higher order so as to obtain a new maximum number set of such generators the latter operators generate the same group when adjoined to the given Sylow subgroup as the former.

The given method requires only slight modifications to apply to the general case. After a Sylow subgroup whose order is a power of the smallest prime which divides the order of G has been found the adjoined operators of the maximum number set of independent generators, if it contains such operators, should be so selected as to obtain a group of one of the smallest orders possible. The same is true as regards the additional operators thus selected until we obtain a group which involves a Sylow subgroup whose order is a power of the next to the smallest prime number which divides the order of G . After this is done we proceed similarly with the selection of

additional independent generators of G . This proves that *whenever a group contains at least one maximum number set of independent generators all of its Sylow subgroups are abelian and of type 1^k and each of its Sylow subgroups is invariant under all of its Sylow subgroups whose orders are powers of smaller prime numbers.* In particular, it contains only one Sylow subgroup whose order is a power of the largest prime which divides the order of the group.

An interesting special case presents itself when G contains a maximum number set of independent generators which is composed of operators of order 2. In this case the Sylow subgroup whose order is a power of 2 is selected just as the Sylow subgroup whose order is a power of the smallest prime which divides the order of G is selected in the general case. If the group contains additional operators it may be extended so as to obtain a group whose order is divisible by a larger prime number. The operators of the cyclic group generated by an operator whose order is equal to this prime number are transformed either into themselves or into their inverses by all the operators of the given Sylow subgroup and hence they are invariant under a subgroup of index 2 contained in this Sylow group if they are not invariant under this entire group. As an operator of odd prime order cannot be transformed into its inverse by such an operator it results that these operators are relatively commutative under G . Hence the following theorem: *If a group has a maximum number set of independent operators composed of operators of order 2 then its operators of odd prime order generate an abelian invariant subgroup.*

From the fact that the independent generators of such a group can be so selected that each of them is of prime order it results that when a group has a maximum number set of independent generators the order of none of its operators is divisible by the square of a prime number. When such a set is composed of operators of order 2 the invariant abelian subgroup generated by its operators of odd prime order can usually be extended in various ways so as to obtain the entire group. The smallest such group results when we adjoin to this subgroup an operator of order 2 which transforms each operator of this subgroup into its inverse. If k is the sum of the number of independent generators in the Sylow subgroups of this abelian subgroup of odd order then the number of the independent generators of G composed of operators of order 2 is $k + 1$ whenever G is this smallest extended group. It is $2k$ when G is the largest possible such extended group based on the given abelian group generated by operators of odd prime order. The objects of the present article are extensions and clarifications of results announced in an article which appeared in the preceding volume of these PROCEEDINGS, pages 333-337.

SPECIAL HOMOLOGY GROUPS

By M. RICHARDSON

INSTITUTE FOR ADVANCED STUDY

Communicated December 3, 1937

In this note, new invariants of the topological type of a finite group of homeomorphisms are introduced.

1. Let K be a simplicial n -complex and G a finite group of simplicial homeomorphisms $T_0 = 1, T_1, \dots, T_{m-1}$ of K on itself. By an *invariant subdivision* K' of K is meant a simplicial subdivision such that (a) the T_i permute the simplexes of K' and (b) no vertex v is adjacent to two distinct transforms $T_i v' \neq T_j v'$ of any vertex v' unless $v = T_i v'' = T_j v''$. Such subdivisions K', K'', \dots exist. Let K denote hereafter a definite invariant subdivision.

Let a_0, a_1, \dots, a_{m-1} be an arbitrary but fixed set of m integers, not all zero, and define the operator a as follows:¹

$$a = a_0 T_0 + a_1 T_1 + \dots + a_{m-1} T_{m-1}. \quad (1)$$

A p -chain C_p (over any coefficient group \mathfrak{G}) is said to be of *type a* if there exists a p -chain X_p such that $C_p = aX_p$. The p -chains of type a form a subgroup a_p of the group L_p of all p -chains of K . The p -cycles of type a form a subgroup \mathfrak{A}_p of the group Γ_p of all p -cycles of K ; in fact $\mathfrak{A}_p = a_p a \cap \Gamma_p$. If C_{p+1} and C_p are both of type a and $FC_{p+1} = C_p$, we write $C_p \stackrel{a}{\sim} 0$ (read a -homologous to zero). Such relations are called² *special homologies* (of type a). The p -chains which are a -homologous to zero form a subgroup \mathfrak{A}_p^0 of the group Γ_p^0 of bounding p -cycles; in general, \mathfrak{A}_p^0 is a proper subgroup of $\Gamma_p^0 \cap \mathfrak{A}_p$. The group $H_p^a(K, G) = \mathfrak{A}_p - \mathfrak{A}_p^0$ is called the *p -dimensional special homology group of type a* over \mathfrak{G} . There is a group $H_p^a(K, G)$ for each choice of the integers a_0, \dots, a_{m-1} . In the special case where $a_0 = 1$ and $a_i = 0$ for $i \neq 0$, $H_p^a(K, G)$ is merely the ordinary homology group $H_p(K)$ over \mathfrak{G} . In general, $H_p^a(K, G)$ is not isomorphic to any subgroup of $H_p(K)$. We have, however, the following theorem: if every p -cycle of type a which bounds is a -homologous to zero (that is, if $\mathfrak{A}_p^0 = \Gamma_p^0 \cap \mathfrak{A}_p$), then $H_p^a(K, G)$ is isomorphic to a subgroup of $H_p(K)$.

It is clear that many other types of special homology can be introduced. For example, suppose K^* is the subcomplex of K composed of all points which are fixed under all the T_i . A chain C_p is said to be of type α if there exists a chain X_p such that $C_p = aX_p \bmod K^*$. We can then define special homology groups $H_p^\alpha(K, G)$ of type α in an obvious way.

There are many algebraic relations among the various groups considered which we shall not state here. If we know the automorphisms of the groups L_p induced by the T_i , we can calculate the special homology groups.

2. The special homology groups over any \mathfrak{G} may be considered as the corresponding homology groups of certain abstract complexes associated in an obvious way with K and the transformations T_i . Abstract complex is meant in a sense slightly more general than that of Mayer;³ that is, a sequence of abelian groups (here, the groups of chains of a particular type) with a boundary homomorphism F such that $FF = 0$. The special homology groups over the integers may be regarded as the corresponding homology groups of abstract complexes, in the sense of Tucker,⁴ whose "cells" are the elements of independent bases for the groups of chains of the particular type considered. For example, denote by s the operator $\sum T_i$; that is, let all $a_i = 1$ in (1). Then the special homology group $H_p^s(K, G)$ of type s over the integers may be considered as the p -dimensional homology group of an abstract complex, in the sense of Tucker,⁴ which is isomorphic with the complex k obtained by identifying all points of K which are congruent under the transformations T_i ; hence $H_p^s(K, G) \cong H_p(k)$.

It was proved⁵ that the automorphisms induced in the $H_p(K)$ by the T_i suffice to determine the Betti numbers of k over the integers or the integers mod π^n , π a prime not dividing the order⁶ m of G . A simple example shows, however, that the automorphisms induced by the T_i in the $H_p(K)$ and in the fundamental group of K together do not determine the torsion group of k , and hence do not determine completely the group $H_p^s(K, G)$.

3. Clearly, continuity considerations have not entered up to this point; hence everything said so far would apply equally well if K were merely an abstract simplicial complex, say. The following theorem gives topological significance to the special homology groups.

THEOREM. *The special homology groups are invariants of the topological type of G .*

This means that if K^0 admits a group G^0 of simplicial homeomorphisms T_i^0 and if there exists a homeomorphism τ mapping K on K^0 such that $T_i^0 = \tau T_i \tau^{-1}$ for all i , then the special homology groups $H_p^i(K, G)$ and $H_p^i(K^0, G^0)$ are isomorphic, for any type i .

Various known proofs of the invariance of the homology groups can be so modified as to yield proofs of this theorem. In particular, the special homology groups can be invariantly characterized either by using singular chains of type i or by using a sequence of "invariant" coverings (that is, coverings whose nerves are invariant in the sense of §1) composed of the stars of the vertices of successive invariant subdivisions with meshes converging to zero. Invariant coverings in general can also be used to apply these ideas to abstract spaces by means of the homology theory of Čech. In this connection, cf. a forthcoming paper of P. A. Smith in the *Annals of Math.*

¹ All expressions of the form (1) where the a_i range independently over all integers constitute the group-ring of G which has been used in recent work of K. Reidemeister, W. Franz and G. de Rham (cf. de Rham, *Matem. Sbornik, N. S. I*, 737-742 (1936) for references). Their work, however, appears to have little or nothing in common with this note.

² The notion of special homology was introduced for the special case where G is of order 2 and (\mathfrak{g}) is the group of integers mod 2 by P. A. Smith, *Proc. Nat. Acad. Sci.*, 19, 612-618 (1933).

³ W. Mayer, *Monatshefte Math. Physik*, 36, 1-42 (1929). The more general definition used here will be exploited in detail by Mayer in a forthcoming set of notes.

⁴ A. W. Tucker, *Ann. Math.*, 34, 191-243 (1933).

⁵ M. Richardson, *Duke Math. Jour.*, 1, 50-69 (1935); Correction, *Ibid.*, 3, 382 (1937).

⁶ For the case where G is generated by a transformation of period m , the homology groups over the integers mod m of k , of K^* , and of k mod the subset k^* of k corresponding to K^* will be discussed in a joint paper by P. A. Smith and the author to appear elsewhere. Certain special homology groups play an important rôle in that paper. The case $m = 2$ was treated by P. A. Smith, loc. cit., and by M. Richardson, *Bull. Amer. Math. Soc.*, 41, 528-534 (1935), under certain restrictive hypotheses.

LOG π AND OTHER BASIC CONSTANTS

BY HORACE S. UHLER

SLOANE PHYSICS LABORATORY, YALE UNIVERSITY

Communicated December 1, 1937

Consideration of the possibility of extending the 70-figure part of my recent table of reciprocals of factorials¹ for values of n greater than 369 or of checking discrete higher data in this table indicated that Stirling's asymptotic approximation formula² for $\log \Gamma(n)$ was best adapted to the calculation of isolated values of $n!$ to a large number of significant figures. Thanks to the labors of J. C. Adams,³ S. Z. Serebrennikov⁴ and D. H. Lehmer⁵ the Bernoulli numbers occurring in this formula are known completely as far as n equal to 110. On the other hand, the term $\frac{1}{2} \log 2\pi$ in Stirling's series has set in the past a fairly low limit to the number of figures obtainable because $\log_e \pi$ and $\log_{10} \pi$ are given correctly in the literature to only 48 and 60 decimal places, respectively. In order to raise this limit to a level at which it will probably not constitute for some years to come a hindrance to those whose researches depend upon the existence of very extended numerical data, I decided to calculate the values of $\log_e \pi$ and $\log_{10} \pi$ to nearly 215 decimal places. Since even a single false digit in the published value of a basic constant can cause incalculable loss of time and energy, and since unfortunately the literature of large numbers abounds in erroneous figures, it seems desirable not only to present

briefly the details of the method followed in the present arithmetical investigation but also to emphasize the care and precautions which were taken in the earnest endeavor to obtain reliable results.

The formula underlying the work is

$$\pi = (355/113)(1 - .0003/3533)f \quad (1)$$

It was derived from the approximation expression for π , discovered by Ramanujan,⁶ by the introduction of the correction factor f which makes formula (1) exact. A rough value for $1 - f$ is 347×10^{-18} . Hence the actual computation of $\log_e \pi$ reduced to that of the Napierian logarithms of 71, 113, $1 - .0003/3533$ and f . The evaluation of the first two of these logarithms was arranged to depend primarily upon the logarithms of 2, 3, 5 and 7, since the latter four have been finally computed by Adams⁷ to about 273 places of decimals.

The series

$$\log \frac{p}{q} = 2 \left\{ \frac{p-q}{p+q} + \frac{1}{3} \left(\frac{p-q}{p+q} \right)^3 + \frac{1}{5} \left(\frac{p-q}{p+q} \right)^5 + \dots \right\} \equiv 2S[(p-q)/(p+q)] \quad (2)$$

is very convenient when $p - q = 1$, a condition which is fulfilled by the pairs of numbers (5041, 5040) and (226, 225). Since $5041 = 71^2$ and $5040 = 2^4 \cdot 3^2 \cdot 5 \cdot 7$, the logarithm of 71 was calculated from the equation

$$\log_e 71 = 2 \log_e 2 + \log_e 3 + (\log_e 5 + \log_e 7)/2 + S(1/10081),$$

in which the last term denotes the series between the braces of formula (2) when $p = 5041$ and $q = 5040$. Similarly, letting $p = 226 = 2 \cdot 113$ and $q = 225 = 3^2 \cdot 5^2$, the logarithm of 113 was obtained from the relation

$$\log_e 113 = 2(\log_e 3 + \log_e 5) - \log_e 2 + 2S(1/451).$$

For the factors f (used as $1 - \epsilon$) and $1 - .0003/3533$ it was necessary to employ the series

$$-\log(1 - x) = x + \frac{1}{2} x^2 + \frac{1}{3} x^3 + \dots \quad (3)$$

The noteworthy advantage of Ramanujan's expression is that when the fractions $3/(3533 \times 10^4)$ and ϵ are substituted for x in formula (3) the series converges respectively with satisfactory speed and with great rapidity. Incidentally since by its defining fraction,

$$(113)(3533 \times 10^4)\pi/(355)(35329997),$$

f is directly proportional to π , ϵ is also an interminable decimal fraction which must be carried to a greater number of places than the arbitrary limit (215) set for $\log_e \pi$. Hence the integral powers of ϵ had to be ob-

tained by successive multiplication instead of repeated division as was the case for the series the denominators of whose terms were integral multiples of powers of the finite numbers 451, 3533 and 10081.

It is now appropriate to consider the checks which I have applied both to the data computed by Adams and to the new numbers involved in the present work. Although the probability of finding an error in Adams' celebrated results may be considered as extremely small, nevertheless a careful study of his two original papers on this subject caused me to doubt if he had subjected his final values of $\log_e 3$, $\log_e 5$ and $\log_e 7$ to any direct check. In the equation of condition $a - 2b + c = d + 2e$, which Adams found was satisfied to 274 places of decimals, the letters do not symbolize the logarithms of 2, 3, 5 and 7 but have the following meanings: $a = \log_e(10/9)$, $b = \log_e(25/24)$, $c = \log_e(81/80)$, $d = \log_e(50/49)$ and $e = \log_e(126/125)$. It was possible to make a mistake in solving such linear equations as $\log_e 3 = 11a - 3b + 5c$, $\log_e 7 = 19a - 4b + 8c + e$, etc. Adams' work on Euler's constant, which included the independent computation of $\log_e 500$ and $\log_e 1000$, placed the first 263 figures of $\log_e 2$ in quite a different category. Finally, since I failed to find recorded in the literature any case in which Adams' published data had been used or corroborated, the ever present danger of a typographical error in the higher decimals (above 100) still existed.

Since $4375 = 5^4 \cdot 7$, and $4374 = 2 \cdot 3^7$, formula (2) requires that the condition

$$(4 \log_e 5 + \log_e 7 - \log_e 2 - 7 \log_e 3)/2 - S(1/8749) = 0$$

be fulfilled. The terms of $S(1/8749)$ were calculated to 233 decimal places. Let S_∞ denote the limit $a/(1 - r)$ of a simple geometrical series. In the present case it was found that $S_\infty - S_{30} = 8749/76545000 - S_{30} = 3 \times 10^{-233}$, where S_{30} means the sum of the 30 requisite terms of the series. When Adams' logarithms of 2, 3, 5 and 7, and my value of $S(1/8749)$ were substituted in the equation of condition given above, the left-hand member equaled $1 \cdot 10^{-233}$. A very simple check on all the figures of $\log_e 2$ and $\log_e 3$ was available due to the fact that in August of the year 1900 I finished a longhand computation of π which agreed perfectly with the accepted datum. This earlier work afforded unimpeachable values for the sums of the positive terms and for the sums of the negative terms of both $\tan^{-1}(1/5)$ and $\tan^{-1}(1/239)$. Let $s(+)$ and $s(-)$ denote the arithmetical values of the two sums in the case of $\tan^{-1}[(p - q)/(p + q)]$, then, in the notation introduced above in formula (2),

$$\log(p/q) = 2S[(p - q)/(p + q)] = 2[s(+)] + s(-).$$

For $p = 3$ and $q = 2$ it was found that $\log_e 3 - \log_e 2 - 2S(1/5) = 2.5 \times 10^{-275}$. Since my value of $\tan^{-1}(1/5)$ agreed with that given by Wm.

Rutherford⁸ to 287 places of decimals, the values of $\log_e 2$ and $\log_e 3$ are alone responsible for the resultant error 2.5×10^{-275} which is quite as small as, if not smaller than, Adams' own estimate.

Attention will now be turned to the tests to which the new arithmetical data have been subjected. Throughout the entire work every quotient obtained by machine division was checked at least once by machine multiplication, and the results of mental short-division were gone over on two or three occasions which were far apart in time. The separate terms of the series for $\log_e 71$ were carried to 215 decimal places, and it was found that $S_\infty - S_{27} = 10081/101626560 - S_{27} = 3.2 \times 10^{-216}$. The same approximation was used in the case of $\log_e 113$, and the geometrical progression check yielded $S_\infty - S_{40} = 451/203400 - S_{40} = 3.8 \times 10^{-215}$. This difference is not at all excessive because 40 terms contributed to the figures in the highest decimal places, and certain of the digits occupying the 215th place were increased by unity conformably to the usual convention. The computation of $\log_e(1 - .0003/3533)$ was terminated at the 215th place of decimals, and $S_\infty - S_{30} = 3/35329997 - S_{30} = 0 \times 10^{-215}$. The series for $\log_e(1 - \epsilon)$ converged so rapidly that only 14 terms were required in carrying the calculation to about 222 decimal places. This higher degree of approximation was attained advisedly because experience has taught me to segregate the digits of a very long number into groups of 8 when multiplying with the 10-column Monroe machine, and it was necessary to employ 28 such groups in order to avoid uncertainty about the digits in the 214th and 215th decimal places. The check relation in this case is that $f \cdot (1 + \sum_1^{14} \epsilon^n)$ should equal unity. The value found was $1 + 9 \times 10^{-224}$. Since $\log_e 5$ is known to a still higher degree of approximation, it now follows from formula (1) that the limit of accuracy of my value of $\log_e \pi$ depends only upon the reliability of the final digits of the three numbers $\log_e 71$, $\log_e 113$ and $\log_e(1 - .0003/3533)$.

In September, 1937, I tested anew all phases of the work which were not guaranteed by the different values of S_∞ , more specifically the decimal positions of the leading figures of all the terms of the several secondary series were verified, the terms of these series were multiplied by their respective small integers and the products compared with the corresponding terms of the primary geometric series, etc. No error of any kind was detected.

The last stage of the investigation consisted in transforming from the Napierian system to the base 10 not only my new data but also, for sake of completeness, about 230 figures of the logarithms of 2, 3, 5 and 7, originally computed by Adams and given heretofore only to the base e . This procedure not only enhanced the practical value of the data but it also afforded an additional check on the greater part of the work as a whole.

The act of machine multiplying each of the natural logarithms by the modulus was performed twice. Also the value found for $\log_{10} \pi$ was multiplied by $\log_e 10$, that is, by the reciprocal of the modulus. The number obtained agreed perfectly with $\log_e \pi$, thus showing that the earlier multiplication had been performed correctly, and also that $\log_e 10$ is free from error to the number of decimal figures employed. Next, $\log_{10} \pi$ was computed from the equation

$$\log_{10} \pi = 3 \log_{10} 2 - \log_{10} 3 + (\log_{10} 7 - \log_{10} 5)/2 - MN,$$

where M and N denote respectively the modulus and the single number resulting from the expression

$$2S(1/451) - S(1/10081) - \log_e(1 - \epsilon) - \log_e(1 - .0003/3533).$$

The two calculations of $\log_{10} \pi$ led to identical values inclusive of the 215th place of decimals. It was also found that $\log_{10} 2 + \log_{10} 5 = 1 - 1.5 \times 10^{-232}$.

In conclusion I desire to thank my mathematical colleague, John W. Wrench, Jr., for having called my attention both to the existence of Ramanujan's approximation expression for π and to the obvious relation between the terms of the series for $\tan^{-1} x$ and $1/2 \log (1 + x)/(1 - x)$. He has recently computed, without the aid of a machine, the reciprocal of π to about 254 decimal places. It was checked by me, by performing with the machine the multiplication of his result by π . The product found was $1 + 2.4 \times 10^{-254}$. By courtesy this latest value of $1/\pi$ is incorporated in the table given below. The most extended earlier approximation for $1/\pi$ was calculated to 140 decimal places by G. Paucker.⁹ His value is less than the newest by 298×10^{-140} .

TABLE 1

$1/2 \log_{10} 2\pi =$

0.39908	99341	79057	52478	25035	91507	69595	02099	34102	92127
54937	22240	48052	60276	50681	98859	20712	37319	29123	86972
05056	53359	18953	19647	11174	66092	97915	99471	78837	75112
84289	65464	24863	18097	51691	73867	72077	27707	09582	41828
29137	91498	4112(207)						

$\log_e \pi =$

1.14472	98858	49400	17414	34273	51353	05871	16472	94812	91531
15715	13623	07147	21377	69884	82607	97836	23270	27548	97077
02009	81222	86979	89159	04820	55279	23456	58727	90810	78810
28682	52763	93914	26634	59029	02484	77335	88699	37789	20311
96308	24756	794(0	5)						

$\log_{10} \pi =$

0.49714	98726	94133	85435	12682	88290	89887	36516	78324	38044
24461	34053	49992	49471	12089	55267	46555	47386	46429	12223
69428	58999	23596	43915	12872	53374	62308	48343	60752	16520
99021	80283	46762	10775	69356	85915	70723	39384	75663	65266
29294	04414	2052(870)						

 $\log_{10} 2 =$

0.30102	99956	63981	19521	37388	94724	49302	67681	89881	46210
85413	10427	46112	71081	89274	42450	94869	27252	11818	61720
40684	47719	14309	95379	09476	78811	33523	50599	96923	33704
69557	50645	02964	25419	34026	61819	73431	16029	43501	18390
28981	78582	61715	44395	31861	92904	(62)			

 $\log_{10} 3 =$

0.47712	12547	19662	43729	50279	03255	11530	92001	28864	19069
58648	29865	64030	52291	52783	66112	30429	68355	64761	63015
10464	69276	82520	45893	56296	91422	25227	35129	03437	06071
52940	99332	42951	85317	13574	89785	20429	54767	99178	65255
70688	28821	46923	23368	60689	63825	(7)			

 $\log_{10} 5 =$

0.69897	00043	36018	80478	62611	05275	50697	32318	10118	53789
14586	89572	53887	28918	10725	57549	05130	72747	88181	38279
59315	52280	85690	04620	90523	21188	66476	49400	03076	66295
30442	49354	97035	74580	65973	38180	26568	83970	56498	81609
71018	21417	38284	55604	68138	07095	(37)			

 $\log_{10} 7 =$

0.84509	80400	14256	83071	22162	58592	63619	34835	72396	32396
54065	03634	95371	82534	39902	07916	60661	11627	84748	85733
41424	31007	53543	45586	24160	61706	83618	92508	57975	83788
44207	69978	03073	65406	40944	61350	30544	25266	34969	30535
81510	61615	97219	40630	33871	84801	(1)			

 $\log_2 17 =$

2.83321	33440	56216	08024	95346	17873	12653	55882	03012	58574
47872	97237	73788	22925	75800	93128	09120	94868	03750	29475
18348	26204	71870	57291	39759	28419	46788	36429	97545	65742
02127	12599	13208	07209	04790	76471	68172	51666	60296	60850
69091	96813	96134	51492	95164	19209	44718	69393	25481	33184
68944	45037	58003	15646	0300(9)				

 $\log_{10} 17 =$

1.23044	89213	78273	92854	01698	94328	33703	00075	67378	42504
63973	80368	48234	46940	62257	11818	57956	84670	09846	51395
05485	78524	97989	65012	17473	37431	15114	67405	38882	80051
55012	35617	61522	77017	71733	74305	45360	56161	02806	91916
25780	06320	65897	46206	33195	11359	(4)			

$\log_e 71 =$

4.26267	98770	41315	42132	94545	32513	03409	67595	76526	71056
61081	21425	80202	73515	06824	23036	59662	43324	27263	51335
40946	35034	57211	12385	45755	79963	96882	06266	76523	92546
74923	81006	29748	92487	16955	91222	00442	26858	02841	10267
16204	52238	035(8	7)						

 $\log_{10} 71 =$

1.85125	83487	19075	28609	28294	35035	42913	52704	19901	60039
19762	76649	87113	13708	89795	68141	74999	31707	14353	56346
75473	76927	(6)							

 $\log_e 113 =$

4.72738	78187	12340	56858	21314	93616	02167	20293	30388	70299
37947	40348	03534	35739	05559	05007	08361	90102	49987	20779
62663	12060	94546	99147	97069	13900	69288	41850	81695	35298
33887	03009	48395	69536	80408	87907	81456	08500	82081	61919
52260	86331	124(2	9)						

 $\log_{10} 113 =$

2.05307	84434	83419	72279	52270	28609	44818	47783	83623	62209
73395	15705	38185	81852	95591	02402	68378	23275	09682	93555
33103	21934	(8)							

 $1/\pi =$

0.31830	98861	83790	67153	77675	26745	02872	40689	19291	48091
28974	95334	68811	77935	95268	45307	01802	27005	53250	61719
12145	68545	35159	16073	78582	36922	29157	30575	59348	21463
39967	84584	79933	87481	81551	46155	49279	38506	15377	43478
57924	34795	32338	67247	80483	44725	80236	64760	22844	53995
114(4)									

 $\pi^3 =$

9.86960	44010	89358	61883	44909	99876	15113	53136	99407	24079
06264	13349	37622	00448	22419	20524	30017	73403	71855	22318
24025	91377	40231	44077	77234	81220	30046	72761	06176	77985
19766	09903	99856	20657	56305	71506	04123	28403	28780	80935
27693	42164	93966	65715	19044	53873	52617	79413	82025	82605
81693	41251	5(5)							

This table also contains a new value of $\log_e 17$ which was derived from my old values of $s(+)$ and $s(-)$ for $\tan^{-1}(1/239)$ and which is certainly correct to 273 places of decimals, the limit being set solely by the basic data of Adams. The Napierian logarithms of 71 and 113 were converted to the base 10 only to about 110 decimal places because the transformed numbers are not particularly fundamental. It is interesting to find that H. M. Parkhurst¹⁰ gives 04 and 90 instead of 05 and 75 for the digits in places 101 and 102, respectively, for $\log_{10} 17$ and $\log_{10} 71$.

The last constant included in the table, π^2 , was computed by me to about 262 decimal places in order to test and extend the number given by Serebrennikov¹¹ to 220 places. The first 218 significant figures of the two results were identical; his value exceeded mine by 595×10^{-220} . Because of this agreement the machine multiplication was repeated only from the twenty-seventh "sum of products of pairs of eight-figure groups" to the thirty-third sum.

For the numbers tabulated above, the terminal figures whose accuracy may be at all debatable are enclosed in parentheses.

¹ *Trans. Conn. Acad. Arts Sci.*, 32, 381-434 (1937)

² H. T. Davis, *Tables of the Higher Mathematical Functions*, 1, 180-183 (1933).

³ *Scientific Papers of John Couch Adams*, 1, 426-458 (1896).

⁴ S. Z. Serebrennikov, *Mém. Acad. Imp. Sci. St.-Petersbourg*, 16, No. 10 (1905); *Ibid.*, 19, No. 4 (1906).

⁵ D. H. Lehmer, *Duke Math. Jour.*, 2, 460-464 (1936).

⁶ *Collected Papers of Srinivasa Ramanujan*, P. 35 (1927).

⁷ J. C. Adams, *Proc. Roy. Soc. London*, 42, 22-25 (1887).

⁸ Wm. Rutherford, *Proc. Roy. Soc.*, 6, 274, 275 (1853).

⁹ G. Paucker, *Grunert's Archiv Math. Physik*, 1 (1), 9-11 (1841).

¹⁰ H. M. Parkhurst, *Astronomical Tables Comprising Logarithms from 3 to 100 Decimal Places*, New York (1876).

¹¹ See the second reference under 4.

EINSTEIN SPACES IN A SPACE OF CONSTANT CURVATURE

BY AARON FIALKOW¹

DEPARTMENT OF MATHEMATICS, BROOKLYN COLLEGE

Communicated December 8, 1937

In this note, we announce certain new results which give a classification of the real Einstein spaces² E_n which can be imbedded in a real space of constant curvature S_{n+1} . The proofs of these theorems will be given elsewhere. We do not restrict ourselves to the case where the first fundamental forms of E_n and S_{n+1} are positive definite. We assume that the imbedding is given by the equations

$$y^\alpha = y^\alpha(x^i) \quad \begin{matrix} i = 1, 2, \dots, n. \\ \alpha = 1, 2, \dots, n+1. \end{matrix}$$

where the $y^\alpha(x^i)$ are real functions of class C^3 and the rank of $\left\| \frac{\partial y^\alpha}{\partial x^i} \right\|$ is n . In some cases, we also assume that the principal normal curvatures are real and that none of the lines of curvature of E_n is tangent to a null vector.³ If these last two conditions are fulfilled we call E_n a *proper*

hypersurface of S_{n+1} and call the imbedding a *proper* imbedding. If the first fundamental form of the enveloping S_{n+1} is definite neither of the two exceptional cases mentioned above can occur and therefore every hypersurface is proper. Thus we find *all* the Einstein spaces which are hypersurfaces of a space of constant curvature whose first fundamental form is definite (in particular, of a Euclidean space). These results generalize theorems obtained by previous writers to whom references are given below.

An Einstein space E_n is defined as a Riemann space V_n whose mean curvature λ is a constant at each point. If $n > 2$, λ is a constant throughout the space. The case $n = 2$ presents no problem since every V_2 is also an E_2 . Neither do we consider the case $n = 3$. For every E_3 is an S_3 and the possibility and manner of its imbedding in a given S_4 is well known. We therefore suppose $n \geq 4$. Our main result is given by

THEOREM 1. *Let E_n ($n \geq 4$) be a real Einstein space of mean curvature λ which is a proper⁴ hypersurface of a real space S_{n+1} of constant curvature K_0 . Let the signatures of the first fundamental forms of E_n and S_{n+1} be s and $s + e$, respectively.⁵ If (a) $e\lambda > e(n-1)K_0$, then E_n is an S_n of Riemann curvature K where $eK > eK_0$. Conversely, each E_n of type (a) can be imbedded as a hypersurface of S_{n+1} and has indeterminate lines of curvature and constant normal curvature. (b) $\lambda = (n-1)K_0$, then E_n is an S_n of Riemann curvature K_0 . Each E_n of type (b) can be imbedded either as a totally geodesic or a developable hypersurface of S_{n+1} . (c) $e\lambda < e(n-1)K_0$, then $eK_0 > 0$ and $\lambda = (n-2)K_0$. E_n contains $\infty^{n-p}S_p$ of Riemann curvature $\frac{n-2}{p-1}K_0$ and also, orthogonal to the S_p , ∞^pS_{n-p} of Riemann curvature $\frac{n-2}{n-p-1}K_0$ (p is any fixed integer so that $2 \leq p \leq n-2$).*

The S_p and S_{n-p} are totally geodesic in E_n . Each E_n of type (c) can be imbedded in S_{n+1} . The curves in any S_p or any S_{n-p} are the lines of curvature of E_n . The principal normal curvature of E_n has one constant value on all the S_p and another on all the S_{n-p} .

The result stated in (a) was recently proved by Thomas⁶ and Cartan⁷ subject to the restriction that S_{n+1} is a Euclidean space. The theorem in (b) was proved by Kasner⁸ for a Euclidean S_{n+1} with $n = 4$ and for any dimensionality by Schouten and Struik.⁹ Eisenhart¹⁰ showed that the result is true in any flat space. The existence of Einstein spaces of type (c) is proved here for the first time. The possibility of an E_4 of type (c) in a Euclidean S_5 was first indicated by Kasner.¹¹ He asked the question whether such E_4 actually existed. We answer this question in the negative in

COROLLARY I. *There are no Einstein spaces of dimensionality $n \geq 4$ of negative mean curvature which are hypersurfaces of a Euclidean space.*

There is an analogous theorem for spaces of constant negative curvature whose first fundamental form is positive definite. Another immediate consequence of Theorem 1 is

COROLLARY II. *Every Einstein space of dimensionality $n \geq 4$ which is a proper⁴ hypersurface of a flat space is a hypersphere, hyperplane or developable hypersurface.*

The first fundamental form and the equations of the imbedding of an E_n of types (a) or (b) in an S_{n+1} are known. We now state the corresponding results for type (c).

COROLLARY III. *The first fundamental form of an E_n of type (c) may be written as*

$$ds^2 = \frac{e_1(dx^1)^2 + \dots + e_p(dx^p)^2}{\left[1 + \frac{K_1}{4}(e_1x^{1^2} + \dots + e_px^{p^2})\right]^2} + \frac{e_{p+1}(dx^{p+1})^2 + \dots + e_n(dx^n)^2}{\left[1 + \frac{K_2}{4}(e_{p+1}x^{p+1^2} + \dots + e_nx^{n^2})\right]^2}$$

where $K_1 = \frac{n-2}{p-1} K_0$, $K_2 = \frac{n-2}{n-p-1} K_0$ and each e is $+1$ or -1 .

THEOREM 2. *An E_n of type (c) in an S_{n+1} is indeformable and is imbedded in the S_{n+1} by means of the algebraic equations*

$$e_1z^{1^2} + \dots + e_{p+1}z^{p+1^2} = \frac{p-1}{n-2} \cdot \frac{1}{K_0}$$

$$e_{p+2}z^{p+2^2} + \dots + e_{n+2}z^{n+2^2} = \frac{n-p-1}{n-2} \cdot \frac{1}{K_0}$$

where the e 's are each $+1$ or -1 and the S_{n+1} is defined by

$$e_1z^{1^2} + \dots + e_{n+2}z^{n+2^2} = \frac{1}{K_0}.$$

Thus an E_n of type (c) is also the intersection of two spherical hypercylinders in a flat space of $(n+2)$ dimensions. For $n=4$, this situation was discussed by Kasner¹² who showed that the E_4 whose first fundamental form is

$$\frac{dx^{1^2} + dx^{2^2}}{\left[1 + \frac{K}{4}(x^{1^2} + x^{2^2})\right]^2} + \frac{dx^{3^2} + dx^{4^2}}{\left[1 + \frac{K}{4}(x^{3^2} + x^{4^2})\right]^2}$$

may be imbedded in a Euclidean S_6 by means of equations analogous to those of Theorem 2.

He also found that this space is the only E_4 whose first fundamental form is the sum of two forms, one involving only p of the variables, the other involving the remaining $4 - p$ variables. When the first fundamental form of a V_n may be written as

$$ds^2 = g_{\alpha\beta}(x^\alpha)dx^\alpha dx^\beta + g_{\gamma\delta}(x^\gamma)dx^\gamma dx^\delta \quad \begin{matrix} \alpha, \beta = 1, 2, \dots, p \\ \gamma, \delta = p + 1, p + 2, \dots, n, \end{matrix}$$

the form is called *separable* and the two forms $g_{\alpha\beta}dx^\alpha dx^\beta$ and $g_{\gamma\delta}dx^\gamma dx^\delta$ are called its *components*. It is known that the subspaces $x^\alpha = \text{constant}$ as well as the subspaces $x^\gamma = \text{constant}$ are totally geodesic in V_n . It follows from Corollary III that the first fundamental form of an E_n of type (c) is separable and each component represents a space of constant curvature. However, if $n > 4$, other E_n 's than those of type (c) exist whose first fundamental form is separable. This follows from

THEOREM 3. *If the first fundamental form of an Einstein space of mean curvature λ is separable, each component is the first fundamental form of an Einstein space of mean curvature λ . If one component involves only one differential, $\lambda = 0$. Conversely, only the first fundamental forms of Einstein spaces are separable in this manner.*

By a repeated application of this theorem, we may obtain an obvious generalization which applies to an E_n whose first fundamental form is separable into more than two components.

Closely related to the problem of finding the actual imbedding of an E_n in an S_{n+1} as considered in the preceding paragraphs is the algebraic characterization of such spaces. In a recent paper, Allendoerfer¹³ gave such a characterization of the E_n 's with $\lambda \neq 0$ which may be imbedded in a flat space. We easily show by his methods that it is possible to obtain a similar characterization of the E_n 's in an S_{n+1} of Riemann curvature K_0 if $\lambda \neq (n-1)K_0$. However, it is open to question whether this algebraic characterization applies to any E_n 's which are not discussed above; i.e., those E_n 's for which the imbedding is not proper. Allendoerfer's proof depends upon a theorem due to Thomas.¹⁴ This theorem states that under certain general conditions, the Codazzi equations for a hypersurface of a flat space are consequences of the Gauss equations. It is easy to show that Thomas' theorem is true even when the enveloping space is not a flat space but any Riemann space.

¹ Most of the results of this paper were obtained while the author was a National Research Fellow at Princeton University and the Institute for Advanced Study.

² We denote an n -dimensional Riemann space, Einstein space and space of constant curvature by V_n , E_n and S_n , respectively.

³ Brinkmann has shown that Einstein spaces E_4 exist whose first fundamental form is indefinite which may be imbedded in a flat space S_4 so that all of the lines of curvature are tangent to null vectors. (Cf. H. W. Brinkmann, "Conformal Mapping of Einstein Spaces," *Math. Ann.*, **94**, 140-141 (1925).)

⁴ The assumption "proper" is not necessary if the first fundamental form is definite.

⁵ Similarly ϵ may be defined for any V_n in a V_{n+1} and is always $+1$ or -1 . The value of ϵ depends upon the character of the unit normal to V_n in V_{n+1} . If the first fundamental form of V_{n+1} is positive definite, $s = n$ and $\epsilon = +1$.

⁶ T. Y. Thomas, "On closed spaces of constant mean curvature," *Amer. Jour. Math.*, **58**, 702-704 (1936).

⁷ T. Y. Thomas, "Extract from a Letter by E. Cartan Concerning My Note: On closed spaces of constant mean curvature," *Amer. Jour. Math.*, **59**, 793-794 (1937).

⁸ E. Kasner, "The Impossibility of Einstein Fields Immersed in a Flat Space of Five Dimensions," *Amer. Jour. Math.*, **43**, 126 (1921).

⁹ J. A. Schouten and D. J. Struik, "On Some Properties of General Manifolds Relating to Einstein's Theory of Gravitation," *Amer. Jour. Math.*, **43**, 215 (1921).

¹⁰ L. P. Eisenhart, *Riemannian Geometry*, 199-200 (1926).

¹¹ E. Kasner, "Geometrical theorems on Einstein's cosmological equations," *Amer. Jour. Math.*, **43**, 219 (1921).

¹² E. Kasner, "An Algebraic Solution of the Einstein Equations," *Trans. Amer. Math. Soc.*, **27**, 103-104 (1925); see *Proc. Nat. Acad. Sci.*, **11**, 95 (1925).

¹³ C. B. Allendoerfer, "Einstein Spaces of Class One," *Bull. Amer. Math. Soc.*, **43**, 265-270 (1937).

¹⁴ T. Y. Thomas, "Riemann Spaces of Class One and Their Characterization," *Acta Math.*, **67**, 191 (1936).

CLASSIFICATION OF ELEMENT TRANSFORMATIONS BY MEANS OF ISOGONAL AND EQUI-TANGENTIAL SERIES

BY EDWARD KASNER AND JOHN DE CICCIO

DEPARTMENT OF MATHEMATICS, COLUMBIA UNIVERSITY AND BROOKLYN COLLEGE

Communicated November 18, 1937

Introduction.—This paper is a continuation of a paper by the senior writer entitled "The geometry of isogonal and equi-tangential series," published in the Transactions of the American Mathematical Society, vol. 42, no. 1, pp. 94-106. This joint paper may be read independently of the preceding paper.

First, we define two simple operations or transformations on the lineal elements of the plane. A *turn* T_α converts each element into one having the same point and a direction making a fixed angle α with the original direction. By a *slide* S_k , the line of the element remains the same and the point moves along the line a fixed distance k .

We term ∞^1 elements to be a *series* of elements; this includes a union (curve or point) as a special case. By applying a turn T_α to the elements of a union, we obtain a series which we call an *isogonal* series. When a slide S_k is applied to the elements of a union, the resulting series is said to be an *equi-tangential* series.

In the earlier paper, it was shown that the totality of all element transformations may be divided into two distinct classes with respect to the number of isogonal (or equi-tangential) series which are carried into isogonal (or equi-tangential) series; namely, 1° —those which carry all ∞^∞ isogonal (or equi-tangential) series into isogonal (or equi-tangential) series. Any transformation of this class is the product of a conformal transformation by a turn (or the product of an equi-long transformation by a magnification by a slide); 2° —those which carry no more than ∞^4 isogonal (or equi-tangential) series into isogonal (or equi-tangential) series. In this paper, it is our purpose to give a more extensive classification of the element transformations with respect to the precise number of isogonal (or equi-tangential) series which are transformed into isogonal (or equi-tangential) series.

UNIONS INTO ISOGONAL SERIES.—THEOREM 1. *All element transformations are classified into four distinct classes with respect to the number of unions which are converted into isogonal series.*

The classes are described as follows:

1° . Those transformations which convert all (∞^∞) unions into isogonal series. Any transformation T of this class is the product of a contact transformation by a turn. These transformations form a group.

2° . Those which convert a single two-parameter (∞^2) family of unions into isogonal series, and no other family of unions of higher or equal number of parameters. Any transformation T of this class is characterized by the following three properties: (a) All points are converted into points; (b) If C is the associated contact transformation (formed by extending the point transformation), the angle θ between the elements $T(E)$ and $C(E)$ is the same for every element E on any point P ; (c) The angle θ is not the same for all points P .

The single two-parameter family of unions which become isogonal series are, of course, the ∞^2 points which become points. Also, for any transformation of this class, there exists a single one-parameter (∞^1) family of unions which become isogonal series which are not all unions.

3° . Those which convert two two-parameter ($2\infty^2$) families of unions into isogonal series, and no other families of unions of higher or equal number of parameters. Any transformation T of this class is such that it carries ∞^3 unions into points, and such that it does not belong to the classes 1° or 2° .

One of the two two-parameter families of unions which become isogonal series is the set of ∞^2 unions which become points. The other two-parameter family of unions become isogonal series (not all unions).

4° . Those which convert one three-parameter (∞^3) family of unions into isogonal series. Any transformation T of this class is characterized by the fact that there do not exist ∞^3 unions which are converted into points. This is the most general case.

ISOGONAL SERIES INTO ISOGONAL SERIES.—THEOREM 2. *All element transformations are classified into four distinct classes with respect to the number of isogonal series which are transformed into isogonal series.*

These classes are characterized as follows:

1°. Those which convert all (∞^∞) isogonal series into isogonal series. Any transformation T of this group is the product of a conformal transformation by a turn.

2°. Those which convert two two-parameter (2×2) families of isogonal series into isogonal series, and no other family of isogonal series of higher or equal number of parameters. Any transformation T of this class is characterized by the following four properties: (a) All points are transformed into points; (b) The associated contact transformation C of T is conformal; (c) The angle θ between the directions of $C(E)$ and $T(E)$ is the same for all elements E on any point P ; (d) The angle θ is not the same for all points P .

One of the two two-parameter families of isogonal series which become isogonal series is the set of ∞^2 points which are transformed into points. The other two-parameter family of isogonal series become isogonal series (not all points).

3°. Those which convert a single three-parameter (∞^3) family of isogonal series into isogonal series, and no other family of isogonal series of higher or equal number of parameters. Any transformation T of this class is such that it converts the ∞^2 points into points and such that it does not belong to the classes 1° or 2°.

4°. Those which convert a single four-parameter (∞^4) family of isogonal series into isogonal series, and no other family of higher or equal number of parameters. Any transformation of this class is such that it does not transform the ∞^2 points into points.

UNIONS INTO EQUI-TANGENTIAL SERIES.—THEOREM 3. *All element transformations are classified into four distinct classes with respect to the number of unions which become equi-tangential series.*

These classes are:

1°. Those which convert all (∞^∞) unions into equi-tangential series. Any transformation T of this group is the product of a contact transformation by a slide.

2°. Those which convert a single two-parameter (∞^2) family of unions into equi-tangential series, and no other family of unions of higher or equal number of parameters. Any transformation T of this class is characterized by the following three properties: (a) All straight lines are transformed into straight lines; (b) If C is the associated contact transformation, the distance d between the points of $T(E)$ and $C(E)$ is the same for all elements E on any line l ; (c) The distance d is not the same for all straight lines.

If any transformation T of this class is such that the distance d is the

same for all parallel straight lines, then the only unions which become equi-tangential series are the ∞^2 straight lines which are transformed into straight lines. If any transformation T of this class does not possess the preceding property, then there also exists a single one-parameter family of unions (not straight lines) which are transformed into equi-tangential series (not all unions).

3°. Those which convert two two-parameter ($2\infty^2$) families of unions into equi-tangential series, and no other family of unions of higher or equal number of parameters. These transformations are such that they transform ∞^2 unions into straight lines and such that they do not belong to the classes 1° or 2°.

Of course, one of the two-parameter families of unions which become equi-tangential series is the set of ∞^2 unions which are transformed into straight lines. The other two-parameter family of unions are carried into equi-tangential series which are not all unions.

4°. Those which convert a single three-parameter (∞^3) family of unions into equi-tangential series, and no other family of unions of higher or equal number of parameters. Any transformation T of this class is such that it transforms no set of ∞^2 unions into straight lines.

EQUI-TANGENTIAL SERIES INTO EQUI-TANGENTIAL SERIES.

—THEOREM 4. *The element transformations are classified into five distinct classes with respect to the number of equi-tangential series which become equi-tangential series.*

These classes are defined as follows:

1°. Those which convert all (∞^∞) equi-tangential series into equi-tangential series. Any transformation T of this group must be the product of an equi-long transformation by a magnification by a slide.

2°. Those which convert a single two-parameter (∞^2) family of equi-tangential series into equi-tangential series, and no other family of equi-tangential series of higher or equal number of parameters. Any transformation T of this class is characterized by the following five properties: (a) All the straight lines are transformed into straight lines; (b) If C is the associated contact transformation, then under C the distance between the points of every two elements on any line l is magnified by the same quantity m ; (c) The quantity m is the same for all parallel straight lines; (d) The distance between the points of $C(E)$ and $T(E)$ is the same for all parallel elements; (e) The transformation T does not belong to the class 1°.

For any transformation of this class, the only equi-tangential series which become equi-tangential series are the ∞^2 straight lines which are carried into straight lines.

3°. Those which convert two two-parameter ($2\infty^2$) families of equi-tangential series into equi-tangential series, and no other family of equi-tangential series of higher or equal number of parameters. Any trans-

formation T of this class is characterized by the following four properties: (a) All straight lines become straight lines; (b) If C is the associated contact transformation, then C converts all parallel straight lines into parallel straight lines; (c) The distance d between the points of $T(E)$ and $C(E)$ is the same for all elements E on any straight line l ; (d) The transformation T does not belong to the classes 1° or 2° .

One of the two-parameter families of equi-tangential series which are converted into equi-tangential series are the ∞^2 straight lines which are converted into straight lines. The other two-parameter family of equi-tangential series become equi-tangential series which are not unions.

4° . Those which convert a single three-parameter (∞^3) family of equi-tangential series into equi-tangential series, and no other family of equi-tangential series of higher or equal number of parameters. The transformations of this class are such that they carry the ∞^2 straight lines into straight lines and such that they do not belong to the classes 1° , 2° or 3° .

5° . Those which convert a single four-parameter (∞^4) family of equi-tangential series into equi-tangential series, and no other family of equi-tangential series of higher or equal number of parameters. Any transformation T of this class is such that it does not convert the ∞^2 straight lines into straight lines.

The first class in theorems 2 and 4 forms a group, and the intermediate classes obey the condition of involution (Poisson bracket vanishes). The possible sets are ∞^∞ , ∞^4 , ∞^3 , $2\infty^2$, ∞^2 . If, however, we allow for geometric degenerate duplications, the possibilities are ∞^∞ , ∞^4 , in theorems 2 and 4, and ∞^∞ , ∞^3 in theorems 1 and 3.

ABSTRACT SELF-ADJOINT BOUNDARY CONDITIONS

BY J. W. CALKIN

THE INSTITUTE FOR ADVANCED STUDY

Communicated December 1, 1937

In studying the applications of the theory of Hilbert space to partial differential operators of the form

$$L = -\frac{\partial}{\partial x} p(x, y) \frac{\partial}{\partial x} - \frac{\partial}{\partial y} p(x, y) \frac{\partial}{\partial y} + q(x, y),$$

we have considered the following problem: given a bounded set E in the (x, y) -plane with a smooth curve C as boundary, and the functions $p(x, y)$ and $q(x, y)$ defined and appropriately restricted on E , to characterize those boundary conditions on a function and its normal derivative at

C which define domains on which L is a maximal symmetric transformation or has a maximal symmetric closure in the Hilbert space $\mathfrak{L}_2(E)$. The nature of the results of the indicated investigations,¹ together with results in the theory of ordinary differential operators,² suggested strongly the possibility of formulating and discussing a problem in abstract terms, which would have as one realization that given above and as others, similar problems associated with a wide variety of differential operators both ordinary and partial. It is the purpose of the present note to formulate such a problem and to describe briefly the more salient features of its solution.

We employ, with minor modifications, the notation and terminology of Stone.³ We make fundamental use of the theory of transformations between Hilbert and unitary spaces⁴ and of the notion of the graph of a transformation.⁵ The domain, range and graph of a transformation T will be denoted by $\mathfrak{D}(T)$, $\mathfrak{R}(T)$ and $\mathfrak{B}(T)$, respectively. We use the notation $T\mathfrak{N}$ to mean the set in the range of T into which T takes the set \mathfrak{N} in its domain. Although we have occasion to consider simultaneously inner products formed in various spaces, we use the notation $(\ , \)$ for all such functions; the context indicates clearly in every case the space on which the product is formed.

DEFINITION 1. Let H be a closed symmetric transformation in the Hilbert space \mathfrak{S} . A linear transformation A with domain in the graph $\mathfrak{B}(H^*)$ and range in a unitary or Hilbert space \mathfrak{M} is called a reduction operator for H^* if it is closed, has domain dense in $\mathfrak{B}(H^*)$, range dense in \mathfrak{M} , and if the orthogonal complement in the space $\mathfrak{S} \oplus \mathfrak{S} \oplus \mathfrak{M}$ of $\mathfrak{B}(A)$ is the set of all vectors of the form $\{H^*f, -f, WA\{f, H^*f\}\}$, where W is a unitary transformation in \mathfrak{M} such that $W^2 + I \equiv O$.

It is readily proved that $\mathfrak{D}(A) \supseteq \mathfrak{B}(H)$ and that $A\{f, H^*f\} = 0$ if and only if f is in the domain of H . The case that H is self-adjoint and $A \equiv O$ is clearly trivial; hereafter we exclude it.

We denote by \mathfrak{M}^+ and \mathfrak{M}^- the characteristic manifolds of W for the characteristic values i and $-i$, respectively. Evidently $\mathfrak{M}^- = \mathfrak{M} \ominus \mathfrak{M}^+$.

For simplification we write merely Af for $A\{f, H^*f\}$. Thus we have the abstract Lagrange identity

$$(f, H^*g) - (H^*f, g) + (Af, WAg) = 0 \quad (1)$$

for all f and g for which Af and Ag are defined.

We describe now a general linear boundary condition associated with the transformation H^* of Definition 1.

DEFINITION 2. If \mathfrak{N} is a linear manifold in \mathfrak{M} , $\mathfrak{A}(\mathfrak{N})$ is the subset of elements f of $\mathfrak{D}(H^*)$ such that Af is defined and is in \mathfrak{N} . $H(\mathfrak{N})$ is the contraction of H^* with domain $\mathfrak{A}(\mathfrak{N})$. The requirement $Af \in \mathfrak{N}$ is called the boundary condition defining $H(\mathfrak{N})$. If the equation $Af = h$ does not have a

solution in $\mathfrak{D}(H^*)$ for a dense set of elements h of \mathfrak{N} , the boundary condition $Afe\mathfrak{N}$ is said to be degenerate; otherwise it is said to be non-degenerate.

In order to characterize those extensions $H(\mathfrak{N})$ of H which are symmetric, we introduce

DEFINITION 3. A linear manifold \mathfrak{N} in \mathfrak{M} is said to be W -symmetric if $W\mathfrak{N} \subseteq \mathfrak{M} \ominus \mathfrak{N}$. If \mathfrak{N} has no proper W -symmetric extension, it is said to be maximal W -symmetric. If $W\mathfrak{N} = \mathfrak{M} \ominus \mathfrak{N}$, \mathfrak{N} is said to be hypermaximal W -symmetric.

It is clear from the formula (1) that the operator $H(\mathfrak{N})$ of Definition 2 is a symmetric extension of H if \mathfrak{N} is W -symmetric and that every linear symmetric extension S of H such that $\mathfrak{B}(S) \subset \mathfrak{D}(A)$ can be characterized by a boundary condition $Afe\mathfrak{N}$ where \mathfrak{N} is linear W -symmetric. For a complete discussion of the nature of the manifolds \mathfrak{N} for which $H(\mathfrak{N})$ or $\tilde{H}(\mathfrak{N})$ is maximal symmetric, the information provided by the following three theorems is essential.

THEOREM 1. There is a one-to-one correspondence between the class of linear W -symmetric manifolds \mathfrak{N} in \mathfrak{M} and the class of all isometric transformations V with domains in \mathfrak{M}^+ and ranges in \mathfrak{M}^- ; \mathfrak{N} and V correspond if and only if $\mathfrak{N} = \mathfrak{N}(I - V)$. $\mathfrak{N}(I - V)$ is maximal W -symmetric if and only if $\mathfrak{D}(V) = \mathfrak{M}^+$ or $\mathfrak{R}(V) = \mathfrak{M}^-$, and hypermaximal W -symmetric if and only if both conditions are satisfied;

$$\mathfrak{M} \ominus \mathfrak{N}(I - V) = W\mathfrak{N}(I - V) \oplus (\mathfrak{M}^+ \ominus \mathfrak{D}(V)) \oplus (\mathfrak{M}^- \ominus \mathfrak{R}(V)).$$

THEOREM 2. Let U be the transformation in $\mathfrak{S} \oplus \mathfrak{S} \oplus \mathfrak{M}$ which takes $\{f, g, h\}$ into $\{g, -f, Wh\}$. Then $U^2 + I \equiv 0$ and $\mathfrak{B}(A)$ is hypermaximal U -symmetric in $\mathfrak{S} \oplus \mathfrak{S} \oplus \mathfrak{M}$.

THEOREM 3. If \mathfrak{M} has a finite dimension number or if A is bounded, the range of A is the entire space \mathfrak{M} .

It is clear from Theorem 3 that, when \mathfrak{M} is a unitary space or when A is bounded, no boundary condition $Afe\mathfrak{N}$ is degenerate and that every linear contraction of H^* is characterized by a boundary condition. Moreover it is readily proved by use of Theorem 3 that $H(\mathfrak{N})$ is maximal symmetric (self-adjoint) if and only if \mathfrak{N} is maximal (hypermaximal) W -symmetric. A deeper analysis of the situation, involving study of the isometric operator associated with $\mathfrak{B}(A)$ by Theorems 2 and 1, leads to the following more precise result.

THEOREM 4. If \mathfrak{M} is a unitary space or A is bounded, there is a one-to-one correspondence between the class of all closed symmetric extensions S of H and the class of all closed W -symmetric manifolds \mathfrak{N} in \mathfrak{M} ; S and \mathfrak{N} correspond if and only if $S \equiv H(\mathfrak{N})$ and $S^* \equiv H(\mathfrak{M} \ominus W\mathfrak{N})$. If $\mathfrak{N} = \mathfrak{N}(I - V)$, the deficiency-index of $H(\mathfrak{N})$ is (m, n) , where m and n are the dimension numbers of the manifolds $\mathfrak{M}^- \ominus \mathfrak{R}(V)$ and $\mathfrak{M}^+ \ominus \mathfrak{D}(V)$, respectively. In particular, if j and k are the respective dimension numbers of \mathfrak{M}^+ and \mathfrak{M}^- , (k, j) is the deficiency-index of H .

In the case that A is unbounded, examples can be constructed in which self-adjoint extensions S of H exist such that $\mathfrak{B}(S) \subset \mathfrak{D}(A)$, while neither $A\mathfrak{B}(S)$ nor its closure is maximal W -symmetric. On the other hand, we can also construct examples in which \mathfrak{N} is a closed hypermaximal W -symmetric manifold and the equation $Af = h$ has a solution f in $\mathfrak{D}(H^*)$ for every h in \mathfrak{N} , while $H(\mathfrak{N})$ is not closed and $\tilde{H}(\mathfrak{N})$ is not maximal symmetric. Space limitations prevent us from discussing here our complete analysis of the situation in the case that A is unbounded; we shall merely state one theorem which to some extent typifies the sort of results that can be obtained. In the statement of the theorem, the symbol $\mathfrak{D} \oplus \mathfrak{D} \oplus \mathfrak{M}^+$ denotes the manifold of elements $\{0, 0, h_+\}$ of $\mathfrak{S} \oplus \mathfrak{S} \oplus \mathfrak{M}$ such that h_+ is in \mathfrak{M}^+ .

THEOREM 5. *Let A be unbounded. Then \mathfrak{M}^+ and \mathfrak{M}^- have each the dimension number \aleph_0 and the deficiency-index of H is (\aleph_0, \aleph_0) .*

Let Y be the isometric transformation between the characteristic manifolds of U associated with $\mathfrak{B}(A)$ by Theorems 2 and 1. Let $E_{\mathfrak{M}}$ be the projection in $\mathfrak{S} \oplus \mathfrak{S} \oplus \mathfrak{M}$ with range the set of vectors $\{0, 0, h\}$, where h is the general element of \mathfrak{M} . Then $E_{\mathfrak{M}}Y$ is defined everywhere in the manifold $\mathfrak{D} \oplus \mathfrak{D} \oplus \mathfrak{M}^+$. Let F be the transformation with domain \mathfrak{M}^+ such that $E_{\mathfrak{M}}Y\{0, 0, h_+\} = \{0, 0, Fh\}$. Then F is a bounded linear transformation from \mathfrak{M}^+ to \mathfrak{M}^- and the adjoint F^ from \mathfrak{M}^- to \mathfrak{M}^+ exists and has domain \mathfrak{M}^- .*

Let V be an isometric transformation with domain in \mathfrak{M}^+ and range in \mathfrak{M}^- and let $\mathfrak{N} = \mathfrak{N}(I - V)$. Then for $H(\mathfrak{N})$ to be maximal symmetric it is necessary and sufficient that

either $\mathfrak{N}(V - F) \supset A\mathfrak{B}(H(\mathfrak{M}^-))$ or $\mathfrak{N}(V^{-1} - F^) \supset A\mathfrak{B}(H(\mathfrak{M}^+))$.*

For $H(\mathfrak{N})$ to be self-adjoint it is necessary and sufficient that both conditions be satisfied.

The examples previously mentioned attest the significance of Theorem 5. We have also

THEOREM 6. *If A is unbounded, the class of maximal symmetric extensions S of H such that $\mathfrak{B}(S) \subset \mathfrak{D}(A)$ and the class of maximal symmetric extensions S of H such that $S \equiv \tilde{S}_0$, $\mathfrak{B}(S_0) \subset \mathfrak{D}(A)$, $\mathfrak{B}(S) \not\subset \mathfrak{D}(A)$, have each the cardinal number of the continuum.*

In conclusion, we point out that it is possible to define a reduction operator on the graph of the adjoint of an arbitrary closed symmetric transformation H in \mathfrak{S} . One has only to take as \mathfrak{M} the space $\mathfrak{B}(H^*) \ominus \mathfrak{B}(H)$, as A the projection in $\mathfrak{B}(H^*)$ with range \mathfrak{M} .⁶ Application of Theorem 4 to this situation leads again to the familiar method of constructing symmetric extensions of H by means of isometric extensions of its Cayley transform $X \equiv (H + iI)(H - iI)^{-1}$.⁷

The theory here described, together with related results and applications

to both ordinary and partial differential operators, will be discussed in detail in papers now being prepared for publication elsewhere.

¹ Some of the results are briefly described in *Bull. Amer. Math. Soc.*, abstracts 43-3-114, 43-3-209, 43-9-323. There are two errors of statement in the first abstract: on page 171, line 1, the symbols " \subset " should be replaced by " \supset " and on page 171, line 2, " $H(W)$ " should be replaced by " $\tilde{H}(W)$."

² M. H. Stone, *Linear Transformations in Hilbert Space*, New York, 1932, 424-530, especially Theorems 10.7 and 10.18. See also paper of I. Halperin, *Ann. Math.*, 38, 880-919 (1937).

³ *Opus cit.*

⁴ For a discussion of the geometric aspects of the theory of transformations between Hilbert spaces, see F. J. Murray, *Trans. Amer. Math. Soc.*, 37, 301-338 (1936), especially 301-312; the hypothesis that the transformations there considered have domains in a Hilbert space \mathfrak{H}_1 and range in a Hilbert space \mathfrak{H}_2 can be altered, in all of the theorems which we use, to allow either \mathfrak{H}_1 or \mathfrak{H}_2 or both to be unitary.

⁵ J. v. Neumann, *Ann. Math.*, 33, 294-310, especially 299 (1932); Murray, *loc. cit.*, 305-307.

⁶ M. H. Stone has recently communicated to the author a characterization of all reduction operators which can be defined on the graph of the adjoint of a closed symmetric transformation H .

⁷ J. v. Neumann, *Math. Ann.*, 102, 49-131 (1929). See also Stone, *opus cit.*, Chap. IX.

A SIMPLIFIED METHOD FOR AUXIN EXTRACTION

BY J. VAN OVERBEEK

WILLIAM G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated December 13, 1937

When the auxin content of a plant or part of it has to be determined two ways are open for analysis. (1) The oldest one is the *diffusion method*. The auxin of the plant material is allowed to diffuse into agar blocks. This method is more suited for determining the free moving auxin and the auxin production of tips and buds than for determining the actual auxin content of the plant. (2) The *extraction method* was first used by Thimann (1934). He extracted Avena seedlings by crushing them in chloroform and HCl. This procedure was repeated twice. Laibach and Meyer (1935) extracted maize and Helianthus with alcohol. The extract was concentrated and taken up in lanolin. This auxin paste was smeared on one side of intact Avena test plants. In corn plants up to 30 cm. in height no auxin could be found with exception of the coleoptile tips. Boysen Jensen (1937) reported good results with Thimann's method on Phaseolus. He also used ether and acetic acid and dropped the ether extract on agar

blocks (micromethod). Van Raalte (1937) extracted auxin from *Vicia* roots with ether and HCl.

When I tried to extract maize seedlings Thimann's chloroform and HCl method was first used. It failed to give results. Ether and acetic acid gave somewhat better results, but Boysen Jensen's dropping method could not be made to work satisfactorily. When acid was omitted from the extraction procedure, excellent results were obtained with ether extraction. Table 1 shows the increased yield from corn seedlings after omission of acid. It also shows that acid does not increase the amount of auxin extracted from *Avena* seedlings; in one case it even decreased it. The reason for the poor results with acid extraction may be the following. According to Kögl, Erxleben and Haagen Smit (1934) maize contains large amounts of auxin-b. This auxin is destroyed by acid. Another reason for the unsatisfactory preliminary results is the low auxin concentration of corn plants. After a suitable extraction method had been worked out it appeared that the auxin concentration of maize is about one-tenth of that of *Avena*. This was the more surprising since coleoptiles of corn produce large amounts of auxin in their tips.

The extraction method finally arrived at is described below. Its main features are the total omission of acid, the avoidance of crushing the material (considerable labor saving if large numbers of extractions have to be made), one single extraction for a prolonged time (overnight) and the evaporation of the ether extract to complete dryness.

a. Immediately before an extraction was made the ether was purified. Commercial ether was purified twice, recovered ether (see *c*) once. The ether was shaken with FeSO_4 , CaO and water. In the beginning FeSO_4 , H_2SO_4 and water after Brandt (see Weisberger and Proskauer 1935) was used. Then the ether was distilled off. It was found that ether giving a negative benzidine test, a test which is considered extremely sensitive for detecting peroxides, still destroyed a hetero-auxin solution when shaken with it.

b. The plant material was measured (see *i*), cut into the parts that were to be analyzed and put uncrushed in Erlenmeyer flasks. Twenty-five to fifty cc. of ether was added per gram of plant material (less than 25 cc. was never used). This was left in a refrigerator for about 15 hours (overnight). If for some reason the material could not be tested after it had been in ether for 15 hours it was found advisable to evaporate the extract to dryness (see *c* and *d*) and store it in the refrigerator rather than to leave it in the ether. Material stored this way for three or four days had not lost in activity.

c. The ether was next separated from the plant material and from the water. Then it was distilled off (water bath $100^\circ\text{C}.$) until a residue of about 1 or $1\frac{1}{2}$ cc. was left. On account of the explosiveness of the ether

and for economical reasons, no amount larger than one of two cc. was allowed to evaporate in the open.

d. The residue was taken up in a pipet and dropped carefully on the bottom of a small vial (5 cc.) suspended in a beaker with boiling water. In this way the extract was evaporated to complete dryness and deposited at the bottom of the vial.

e. A known amount of agar (3%) was next pipetted on to the dry residue. Mostly 0.5 cc. was used.

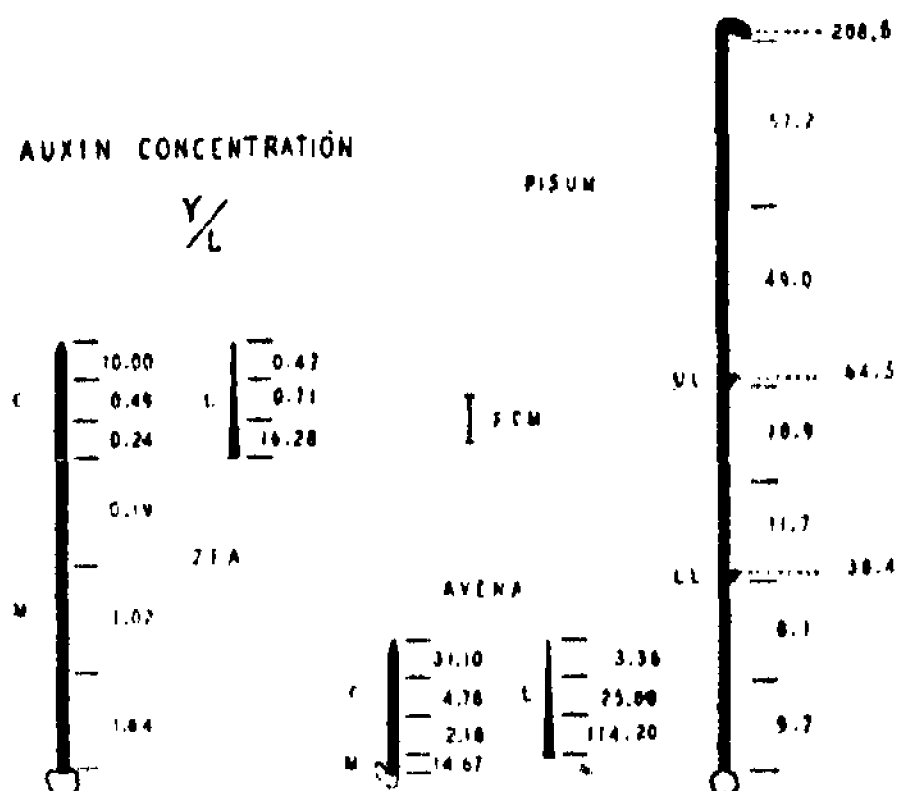


FIGURE 1

Auxin concentrations of etiolated seedlings of corn, oat and pea expressed in gammas hetero-auxin per liter water contained in the plant. It should be noticed that the auxin of the higher plants is of the auxin-a type which has an activity approximately twice that of hetero-auxin. In order to visualize the meaning of the figures it should be remembered that a hetero-auxin concentration of approximately 5 gammas per liter will give a curvature of one degree in the Avena test. Zea plants were 5 days, Avena 3 and Pisum 7 days old, which are stages of development at which these plants are mostly used in the laboratory. C, coleoptile. M, mesocotyl. L, primary leaf. UL, upper lateral bud. LL, lower lateral bud.

At the same time with the blocks to be analyzed a control series was run with a known concentration of hetero-auxin. This was necessary in order to calculate the obtained values in absolute amounts of auxin.

h. If weight determinations had to be made, the lengths of the stems, etc., were measured first, then the fresh weight was determined. Next the material was left for 24 hours in a drying oven, whereupon the water content could be determined.

To secure a thorough mixing of agar and auxin the mixture was stirred and shaken vigorously (the vial still being in the boiling water). When the vial was removed from the boiling water it was left for a few hours for further uniform distribution of the auxin in the agar.

f. The agar auxin mixture was melted again in boiling water and poured into a rectangular brass mold (8 x 10.5 x 1.7 mm.). This mold was cooled by ice for a rapid gelification of the agar. Next this agar plate was divided into 12 equal blocks which were ready now to be put on decapitated oat seedlings for the auxin analysis. The extraction proper is thereby finished but in order to determine the auxin concentration in plants the following additional steps were taken:

g. The Avena test method described by Went and Thimann (1937, pp. 27-51) was employed.

Since the chemistry of auxin is known and hetero-auxin is readily available, it is my opinion that amounts of auxin extracted from or diffused out of plants should be expressed in absolute units rather than in arbitrary ones. The value of the arbitrary units (*A E*, *p u*, unit/cc., *W A E*) varies with the sensitivity of the test plants. An auxin amount expressed in hetero-auxin equivalents on the other hand is independent of the test method, test plant and sensitivity. However, in many cases where relative

amounts of auxin rather than absolute ones are being considered the auxin amounts may be more conveniently expressed in arbitrary units.

The above-described method has been used for the determination of the distribution of the auxin concentrations in maize, *Avena* and pea seedlings and also by Yin (unpublished as yet) to study the auxin distribution of Papaya leaves, and has proved to be reliable and to give reproducible results (*Avena* experiments of table 1). Figure 1 shows the auxin distribution in gammas hetero-auxin equivalents per liter. Note the high auxin concentration in the basal part of the primary leaves of *Avena* and maize and also the relatively high concentration in the basal parts of those seedlings. The concentration in the lateral buds of the pea seedling is higher than that in the adjacent stem tissue. A detailed paper discussing the relation between growth and the auxin distribution in maize and *Avena* seedlings and also the relation between auxin concentration and bud inhibition in the pea seedlings will be submitted to *The Botanical Gazette*.

Boysen Jensen, P., *Planta*, 26, 584 (1937).

Kögl, F., Haagen Smit, A. J., and Erxleben, H., *Zeit. physiol. Chem.*, 228, 90 (1934).

Kögl, F., Erxleben, H., and Haagen Smit, A. J., *Zeit. physiol. Chem.*, 225, 215 (1934).

Laibach, F., and Meyer, R., *Senckenbergiana*, 17, 73 (1935).

Thimann, K. V., *Jour. Gen. Physiol.*, 18, 23 (1934).

Van Raalte, M. H., *Rec. trav. bot. néerl.*, 24, 278 (1937).

Weisberger, A., and Proskauer, E., *Organic Solvents*, Oxford Clarendon Press (1935).

Went, F. W., and Thimann, K. V., *Phytohormones*, Macmillan Company, New York (1937).

LIGHT INTENSITY AND THE NITROGEN HUNGER PERIOD IN THE MANCHU SOYBEAN*

By E. B. FRED, P. W. WILSON AND ORVILLE WYSS

DEPARTMENTS OF AGRICULTURAL BACTERIOLOGY AND AGRICULTURAL CHEMISTRY,
UNIVERSITY OF WISCONSIN

Communicated November 10, 1937

Leguminous plants grown on a nitrogen-poor substrate and dependent on the fixation of atmospheric nitrogen for their supply of this element frequently exhibit during their development a "period of nitrogen hunger." This period occurs fairly early in the growth of the plant, when the stores of nitrogen in the seed have been exhausted and before the centers of fixation, the nodules, have developed sufficiently to meet the ever-increasing demands of the plant for nitrogen. As would be expected the phenomenon usually occurs under environmental conditions which favor photosynthesis,

and the usual evidence of carbohydrate excess are apparent. The plants are stalky with yellow leaves and woody tissues. Ordinarily the period lasts for less than a week, after which the nitrogen fixation process is initiated at a rate that adequately supplies the requirements of the plant for nitrogen. The leaves turn green, and the tissues become more succulent. Thereafter, the rate of carbohydrate synthesis rather than that of nitrogen fixation may become the limiting factor in the development of the plant.

In the summer of 1932 studies were begun at this station for the purpose of comparing the nitrogen metabolism of soybeans which were fixing nitrogen with that of plants which were supplied combined nitrogen. The first experiment was started out-of-doors early in June, just as the prolonged drought of that summer began. The weather during the period immediately following the planting of the soybeans was characterized by sunlight of high intensity and by hot, dry winds. The response of the nodulated soybeans to these rather extreme conditions was most unexpected—they entered the nitrogen hunger period and remained there. Plants supplied NH_4NO_3 developed normally. This difference in the response of the soybean plants to the environmental conditions suggested that the effect was concerned specifically with the nitrogen fixation process. Since the roots of the plants suffering from nitrogen hunger possessed numerous and well-developed nodules, it was concluded that the effect was primarily on the actual fixation of nitrogen. It occurred to us that perhaps the carbohydrate-nitrogen balance in the plant had become so excessive that assimilation of *free* nitrogen was inhibited. To test this hypothesis, part of the nodulated plants which were in the nitrogen hunger stage were shaded for one week in order to decrease carbohydrate formation and to increase the soluble forms of nitrogen in the plant. The response was clear-cut; in a few days nitrogen fixation had begun in the shaded plants, and at the end of a week, the leaves of these plants had become dark green. Analyses for nitrogen confirmed the observation that the shaded plants were markedly superior to the unshaded controls.

As has been indicated in our previous reports,^{1,2,3} these results have important implications for several aspects of the mechanism of symbiotic nitrogen fixation. Among these may be mentioned: (a) the influence of the carbohydrate-nitrogen balance in the host plant on the process, and (b) the relative efficiency of free and combined nitrogen in the nutrition of the soybean. Because of these implications confirmation and extension of the observations are desirable. It should be noted that repetition of this type of work is not entirely in the hands of the experimenter as the requisite first stage, the inhibition of the fixation process, cannot be readily controlled. The weather must be *consistently* "bright and hot" during the first few weeks after planting in order that the nitrogen hunger stage will be prolonged to a point where inhibition of fixation will obtain. If at the

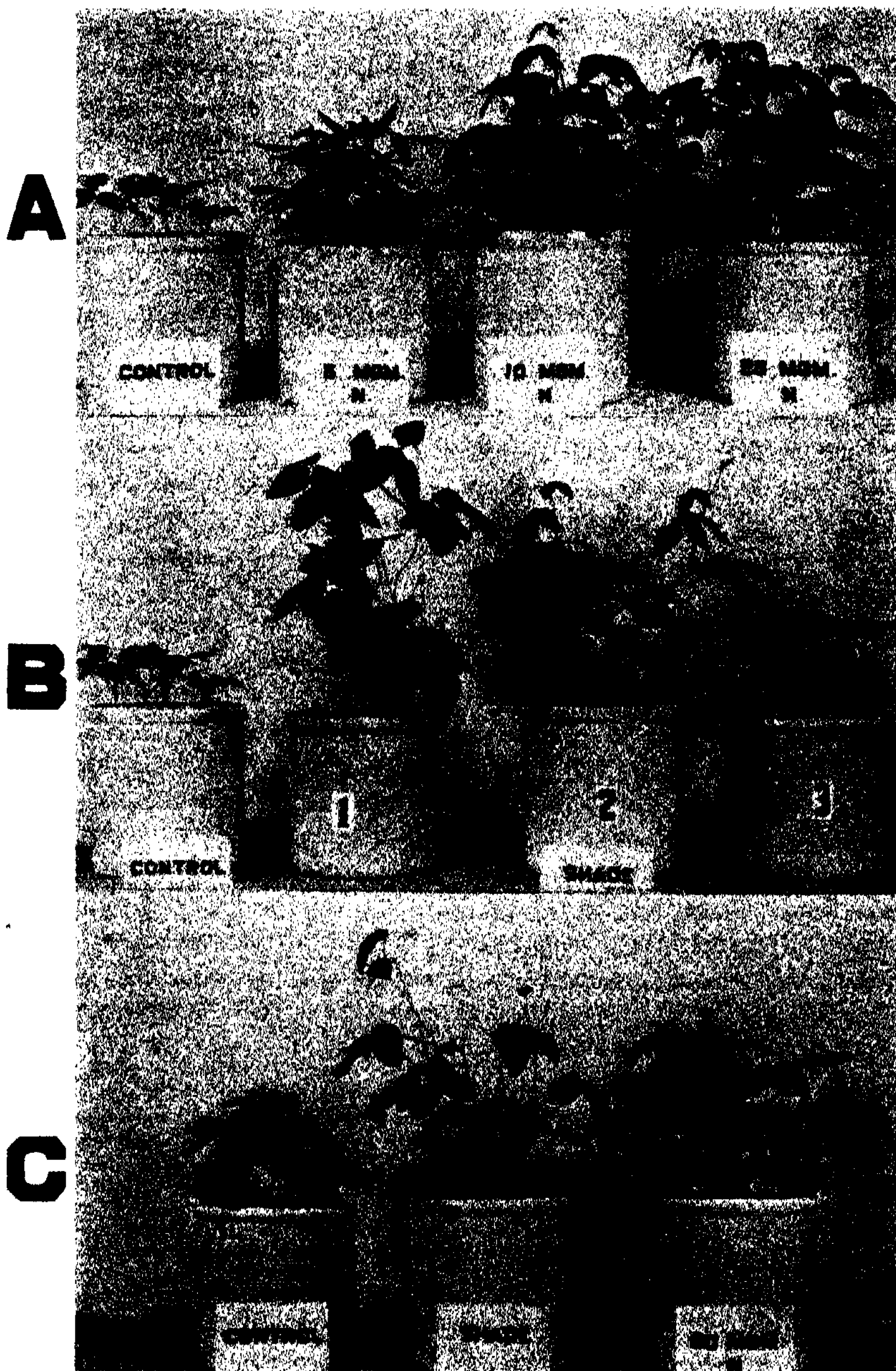


FIGURE 1

(See facing page)

critical stage in the growth of the plant, namely, when the nitrogen in the seed has been utilized, there are a few cool, cloudy days, nitrogen fixation will be initiated, and the further development of the plants is normal. Attempts to duplicate the phenomenon under the more easily controlled environment of the greenhouse with artificial illumination have met with little success, probably because of the low intensity of light available.

To increase the probability of securing plants in which the process of nitrogen fixation has been inhibited, experiments were made during the past two summers in which several jars of soybeans were planted every two weeks during June, July and early August. In 1936 inhibition was observed on two separate occasions, and this inhibition was overcome not only by shading as in our earlier experiments,¹ but also by addition of combined nitrogen. Support was thus obtained for the hypothesis that the inhibition was connected with an excessive carbohydrate-nitrogen relation in the plant. Unfortunately, no analyses were made of these plants, but the experiments were duplicated in 1937 as described in this paper.

Methods.—The methods used were those described previously.¹ Briefly, they consist of growing Manchu soybeans inoculated with an efficient strain of *Rhizobium japonicum* in two-gallon jars on a nitrogen-poor pit sand to which has been added an adequate supply of all plant nutrients except nitrogen. The plants are kept out-of-doors, protected from rain whenever necessary, and are watered daily with nitrogen-free tap water.

Experiment I.—On July 1, 1937, eight jars were planted and inoculated. The first signs of nitrogen hunger were noted about July 15; on July 28 the plants were still in the nitrogen hunger stage and were exhibiting pronounced signs of carbohydrate excess. On this date treatment was begun as follows: two jars were retained as controls; two jars were removed to a shaded cold-frame in which the light intensity was about one-fifth that of the cold-frame in the open; combined nitrogen was added to the remaining six jars. The plants which were shaded or to which combined nitrogen was added soon responded to these treatments. At the end of a week, their

EXPLANATION OF FIGURE

Effect of shading and of combined nitrogen on nodulated soybeans in which an excessive carbohydrate-nitrogen balance had inhibited the nitrogen fixation process.

- A—Experiment I. Control: no treatment; to others combined nitrogen added, as $\text{Ca}(\text{NO}_3)_2$, 33 days before harvest.
- B—Experiment I. { Control: no treatment
1: in shade continuously for 33 days before harvest.
2: in shade for 12 days, returned to sun for 21 days.
3: in shade continuously for 15 days before harvest.
- C—Experiment II. Control: no treatment
Shade: in shade continuously for 19 days before harvest.
50 mg. N: added as $\text{Ca}(\text{NO}_3)_2$, 19 days before harvest.

color had changed from yellow to green, and they had increased in size. After 12 days one of the jars that had been kept in the shade was returned to the sun, inasmuch as the plants were showing the ill effects caused by low light intensity. The two controls in the sun remained yellow and showed no increase in size; on August 15 one of these controls was transferred to the shade. The response of the plants of this control was much slower than that of the first plants transferred, but eventually they also began to turn green and were definitely superior in general appearance to those of the remaining control at harvest on August 30.

Experiment II.—Six jars were planted and inoculated on July 10, 1937. Nitrogen hunger was first apparent at the end of July and continued until

TABLE 1

EFFECT OF SHADING AND OF ADDING COMBINED NITROGEN ON NITROGEN FIXATION BY NODULATED SOYBEANS SUFFERING FROM A PROLONGED NITROGEN HUNGER PERIOD

TREATMENT	NUMBER OF PLANTS	DRY WEIGHT GM.	NITROGEN		
			PER CENT	TOTAL MGM.	FIXED PER PLANT MGM.

<i>Experiment I</i>					
Nodulated control kept in sun	5	4.2	1.14	47.9	2.6
Same + 5 mgm. N as Ca(NO ₃) ₂	6	20.7	1.26	260.5	35.6
Same + 10 mgm. N as Ca(NO ₃) ₂	7	28.5	1.28	364.8	43.7
Same + 25 mgm. N as Ca(NO ₃) ₂	6	30.8	1.45	446.9	63.3
Same + 50 mgm. N as Ca(NO ₃) ₂	8	37.7	1.72	648.1	67.7
In shade continuously for 33 days	6	8.9	2.20	195.8	25.6
In shade for 12 days, returned to sun	6	18.7	1.98	370.0	54.7
In shade for 15 days before harvest	6	6.4	1.08	69.2	4.5

<i>Experiment II</i>					
Nodulated control kept in sun	7	9.0	1.00	90.0	5.9
Same + 50 mgm. N as Ca(NO ₃) ₂	7	20.0	1.60	320.0	31.6
In shade continuously for 19 days	8	18.0	1.21	217.8	20.2

August 11, at which time treatments were started. Two jars were removed to the shade; 50 mgm. nitrogen as $\text{Ca}(\text{NO}_3)_2$ was added to two other jars; and the two remaining jars were retained as controls. These plants were harvested with those of Experiment I on August 30. Since duplicates of the same treatments were identical so far as general appearance was concerned, the plants of only one jar of each treatment were analyzed and the remaining jar after photographing, was used in other work.

Discussion.—Analytical data from the two experiments are summarized in table 1, and the appearance of the plants at harvest is shown in figure 1. The data of table 1 confirm and extend the observations previously reported. They demonstrate that it is unnecessary to remove the plants

from the high light intensity in order to initiate the nitrogen fixation process. Thus they lend support to the hypothesis that an excessive carbohydrate balance in the plant is the inhibitory factor rather than light itself. Furthermore, the breaking of the nitrogen hunger period by use of combined nitrogen indicates that the effect did not arise as a result of lowering the temperature concurrently with the reduction in light intensity. However, the excessive carbohydrate condition which apparently inhibits the nitrogen fixation process may arise in part from high temperatures, as well as from high intensity of light, since it is usually encountered only after periods of hot dry weather. Examination of the nodules of the plants kept in the sun confirmed the observation previously made,¹ namely, that the nodules were well developed and in no way resembled these on plants inoculated with a poor strain of bacteria.

Another point of interest is the difference between the controls (nodulated plants kept in the light with no addition of combined nitrogen) in the two experiments. At the harvest the control plants of Experiment II, although 10 days younger than those of Experiment I, were definitely larger and, in general appearance, were superior. Although the plants of the control jars of Experiment II were still quite yellow in color when harvested, there were some indications that the nitrogen hunger period was being broken by the cloudy weather just preceding the harvest. In contrast the control plants of Experiment I showed no signs of coming out of the nitrogen hunger stage even though they had been exposed to the same weather conditions. The quantitative difference in the behavior of the controls in the two experiments suggests that if the carbohydrate excess is not corrected early, it becomes increasingly difficult to overcome the inhibition. This view received confirmation by the response of those control plants in Experiment I which were transferred to the shade 15 days prior to the harvest. Although at harvest there were definite signs that nitrogen fixation had started in these plants, the inhibition was overcome much more slowly than in the plants which were moved to the shade soon after the nitrogen hunger period had begun.

As previously noted,^{1,2} the *percentage nitrogen* in the plants is only a crude measure of the effective carbohydrate-nitrogen relation in these experiments. Both the plants which were shaded for 15 days in Experiment I and the control plants of Experiment II had a lower percentage nitrogen than did the control plants of Experiment I, in spite of the fact that the development of the latter plants was markedly inferior. The other treatments used, however, caused a definite reduction in the carbohydrate-nitrogen relation, as measured by an increase in the *percentage nitrogen*, coincident with overcoming the inhibition of the nitrogen fixation process.

Shading is extremely effective in breaking the nitrogen hunger period induced by excessive carbohydrates, but if the plants are kept in the shade,

they eventually become fragile and twiny, the usual symptoms in the soybean of inadequate illumination. Once the inhibition is overcome, reduced carbohydrate synthesis causes decreased fixation of nitrogen. If plants are returned to full sunlight after initiation of nitrogen fixation through shading development is normal, and excellent growth and fixation are obtained.

Finally, the quantitative aspects of the combined nitrogen treatments should be emphasized. The greatest fixation of elemental nitrogen was obtained with those plants supplied the largest quantities of combined nitrogen, a rather unusual finding, since combined nitrogen is commonly supposed to inhibit the fixation process.

In our other communications we have emphasized primarily the significance of these findings for theoretical phases of symbiotic nitrogen fixation, but their practical application to problems of agriculture should not be overlooked. Not infrequently experiment stations receive reports of crop failures with soybeans and other leguminous plants which are most puzzling, since apparently all cultural practices known to influence the crop yield, e.g., inoculation of the seed with bacteria of known efficiency, have been properly performed. It is suggested that leguminous plants, when seeded in late spring or early summer, may encounter local environmental conditions which will cause an excessive carbohydrate balance in the host plant and result in inhibition of nitrogen fixation. Our experiments indicate that all the necessary conditions will obtain only on soils of low fertility, especially low with respect to presence of soluble forms of combined nitrogen. Investigation of local weather conditions after seeding of crop may throw light on some of these apparently inexplicable failures.

Summary. When nodulated soybeans are grown under sunlight of high intensity, fixation of atmospheric nitrogen is inhibited, and the nitrogen hunger stage in the plants is unduly prolonged. The inhibition appears to be associated with an excessive carbohydrate-nitrogen balance in the plant, probably with the relation of soluble forms of carbohydrate and nitrogen. Reduction of this excessive carbohydrate-nitrogen relationship either by shading (decrease in photosynthesis and hydrolysis of protein) or by addition of soluble forms of combined nitrogen is accompanied by initiation of the nitrogen fixation process, followed by a normal development of the plant.

* Herman Frasch Foundation in Agricultural Chemistry, Paper No. 146.

¹ Orcutt, F. S., and Fred, E. B., "Light Intensity as an Inhibiting Factor in the Fixation of Atmospheric Nitrogen by Manchu Soybeans," *Jour. Amer. Soc. Agron.*, 27, 550-558 (1935).

² Umbreit, W. W., and Fred, E. B., "The Comparative Efficiency of Free and Combined Nitrogen for the Nutrition of the Soybean," *Jour. Amer. Soc. Agron.*, 28, 548-555 (1936).

³ Wilson, P. W., "The Carbohydrate-Nitrogen Relation in Symbiotic Nitrogen Fixation," *Wis. Agr. Expt. Sta. Bul.*, No. 129 (1935).

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

February 15, 1938

Number 2

Copyright 1938 by The National Academy of Sciences

ORGANISMS REQUIRING VITAMIN B₁

BY WILLIAM J. ROBBINS

DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated December 27, 1937

In earlier articles¹ from this laboratory we have reported that excised tomato roots required an external supply of vitamin B₁ or of thiazole² for continued growth. The pyrimidine alone was ineffective. *Phycomyces Blakesleanus* was found to require vitamin B₁ or both intermediates.³ Neither of the intermediates alone was effective. *Phycomyces nitens* and two species of *Torula*⁴ which we have investigated, *Torula fermentati* and *T. Laurentii*, resemble *Phycomyces Blakesleanus* in requiring an external supply of both intermediates for good growth.

We have found some organisms also which grow well in a medium supplemented with pyrimidine alone, with a mixture of pyrimidine and thiazole, or with vitamin B₁. The thiazole alone is ineffective. Among these organisms are *Torula rosea*, *T. sanguinea*, *Phytophthora jagopyri*, *Schizophyllum commune*, *Sclerotium delphinii*, *S. Rolfsii*, *Sphaerulina trifolii*, *Pythium Butleri* and *P. polycladon*.⁵ In a liquid medium composed of 0.5% MgSO₄·7H₂O, 1.5% KH₂PO₄, 1% or 0.5% Asparagine, 5% or 10% dextrose and certain mineral supplements, these seven organisms grow poorly or not at all. The addition to this medium of 30 units⁶ of pyrimidine, a mixture of 30 units of pyrimidine and 30 units of thiazole or of 30 units of vitamin B₁ permits good growth.

None of the organisms mentioned above can be said to require an external supply of vitamin B₁ since they grow well in a medium lacking vitamin B₁ but supplemented with one or with both of the intermediates.

We have assumed on the basis of evidence presented earlier³ that the vitamin B₁ molecule is the effective agent in determining the growth of *Phycomyces*, and that this organism when grown in a mixture of pyrimidine and thiazole synthesizes the vitamin molecule from the intermediates. Schopfer and Jung⁷ are inclined to believe that the intermediates function as such and that *Phycomyces*, for example, when grown in a solution containing vitamin B₁, splits the vitamin molecule into the intermediates which then play their respective rôles in the metabolism of the organism.

We have found that *Torula rosea*, *T. sanguinea*,⁴ *Phytophthora fagopyri* and *Pythium Butleri*⁵ synthesize thiazole when grown in a medium supplemented with pyrimidine only. Furthermore, excised tomato roots form pyrimidine in a solution supplemented with thiazole only. This shows that some organisms which grow in a medium supplemented with pyrimidine alone or with thiazole alone form the missing intermediate from the elementary substances in the medium. This does not demonstrate that the molecule of vitamin B₁ rather than its thiazole and pyrimidine intermediates is the effective agent. It is, however, a result which would be anticipated on the basis of our assumption.

If we are correct in assuming that the vitamin molecule, as such, is the effective agent it might be anticipated that some of the more highly parasitic fungi may not be capable of synthesizing the vitamin molecule from the intermediates. For such organisms it would not be possible to replace the vitamin by its intermediates. They would require for growth an external source of vitamin B₁.

We have found certain species of *Phytophthora* which apparently require vitamin B₁ and cannot utilize the intermediates satisfactorily. *Phytophthora cinnamomi* and *P. capsici* are of this type. In our experiments these two organisms grew well in a medium of mineral salts and sugar supplemented with vitamin B₁. In the absence of the vitamin or with thiazole, pyrimidine or a mixture of the thiazole and pyrimidine we have secured little or no growth. It is possible that the synthesis of the vitamin from its intermediates is enzymatic and that these organisms lack the necessary enzyme.

Sinclair⁶ and Schopfer and Jung⁷ also have found that the two intermediates will replace the vitamin for *Phycomyces Blakesleanus*. Neither intermediate alone is effective. Schopfer⁹ has reported that *Rhodotorula flava* and *Rhodotorula rubra* are organisms which grow with pyrimidine alone, the thiazole alone is ineffective; and that *Mucor Ramannianus*¹⁰ requires thiazole but not pyrimidine. We are inclined to believe on the basis of the evidence now available that the vitamin molecule is necessary for all of these organisms. Some kinds are capable of synthesizing from the elementary constituents of the medium sufficient for good growth. Others are not. Some synthesize enough of one of the intermediates for good growth but under the conditions of our experiments must be supplied with the other; some synthesize neither of the intermediates in amounts adequate for normal growth but are able to utilize the intermediates if supplied; and some not only do not synthesize either intermediate but are incapable of utilizing them when they are supplied.

The different groups of organisms in their relation to vitamin B₁ and its intermediates are summarized in the following table. The + sign indicates a positive growth effect of the substance given; the — sign, little or no effect.

ORGANISMS	THIAZOLE	PYRIMIDINE	THIAZOLE AND PYRIMIDINE	VITAMIN B ₁
Group I	—	—	—	+
Group II	—	—	+	+
Group III	—	+	+	+
Group IV	+	—	+	+

In Group I are included those organisms which require an external supply of vitamin B₁; for example, *Phytophthora cinnamomi* and *P. capsici*.

Group II includes organisms which require for good growth an external supply of vitamin B₁ or of both intermediates. The thiazole alone or the pyrimidine alone is ineffective. Examples are *Phycomyces Blakesleeanus*, *Phycomyces nitens*, *Torula Laurentii* and *T. fermentati*.

Group III includes those which require an external supply of vitamin B₁, of both intermediates or of pyrimidine. The thiazole alone is ineffective. Examples are *Phytophthora fagopyri*, *Pythium Butleri*, *P. polycladon*, *Schizophyllum commune*, *Sclerotium delphinii*, *Sclerotium Rolfsii* and *Sphaerulina trifolii*.

Group IV includes those which require an external supply of vitamin B₁, of pyrimidine and thiazole, or of thiazole. The pyrimidine alone is ineffective. Examples are *Mucor Ramannianus* and excised tomato roots.

A fifth group might be included comprising organisms which are unaffected by the vitamin or its intermediates in amounts which are effective for the organisms given above. This group includes many saprophytic organisms, for example, *Aspergillus niger*, and perhaps some parasites.

The members of a sixth group (*Rhizopus nigricans*)⁵ are inhibited by amounts of the vitamin or of its intermediates which are favorable for other organisms.

These observations suggest that a biological method of detecting the presence of the vitamin or of its intermediates could be devised. Its success would depend upon the use of suitable organisms and further evidence of the specificity of vitamin B₁ and its intermediates for the organisms. It would also depend in part upon the presence of the vitamin (or intermediates) within certain limits, and the absence of injurious material which would inhibit the growth of the organisms. The scheme presented below has not been used on natural materials and is suggestive only.

I *Phytophthora cinnamomi*

Positive growth effect = vitamin B₁

No growth effect = both intermediates or pyrimidine alone
or thiazole alone or no vitamin B₁ nor
intermediates

See II

II *Phycomyces Blakesleeanus*

Positive growth effect = both intermediates

No growth effect = pyrimidine alone or thiazole alone or no vitamin B₁ nor intermediate See IIIIII *Pythium polycladon*

Positive growth effect = pyrimidine alone

No growth effect = thiazole alone or no vitamin B₁ nor intermediate See IVIV Tomato root or *Mucor Ramannianus*

Positive growth effect = thiazole alone

No growth effect = no vitamin B₁ nor intermediate

¹ William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246-247 (1937). William J. Robbins and Mary A. Bartley, *Proc. Nat. Acad. Sci.*, **23**, 385-388 (1937).

² When the terms, thiazole or pyrimidine, are used in this paper, the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant. These intermediates have been used in the synthesis of vitamin B₁.

³ William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499-502 (1937).

⁴ William J. Robbins and Frederick Kavanagh, *Plant Physiol.* (in press).

⁵ William J. Robbins and Frederick Kavanagh, *Am. Jour. Bot.* (in press).

⁶ A unit is 10⁻⁹ Mole of the compound in question.

⁷ William Henri Schopfer and Albert Jung, *Compt. Rend. Acad. Sci., Paris*, **204**, 1500-1502 (1937).

⁸ H. M. Sinclair, *Nature*, **140**, 361 (1937).

⁹ William H. Schopfer, *Compt. Rend. Acad. Sci., Paris*, **205**, 445-447 (1937).

¹⁰ Werner Müller and William Henri Schopfer, *Ibid.*, **205**, 687-689 (1937).

A CYTOLOGICAL STUDY OF COLCHICINE EFFECTS IN THE INDUCTION OF POLYPLOIDY IN PLANTS

BY O. J. EIGSTI

DEPARTMENT OF BIOLOGY, GREENVILLE COLLEGE

Communicated January 12, 1938

A series of colchicine treatments of plant tissues was begun by the writer as an independent investigation in February, 1937, while employed at the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y. In a report at the annual winter meeting of the A. A. A. S. in 1936, Allen¹ mentioned that colchicine influenced mitotic activity in animal tissue. Soon thereafter Mr. E. L. Lahr,² a colleague in the

Department of Genetics, showed the writer some preparations of animal germinal tissue treated with colchicine previous to killing, in which mitotic figures were more abundant than in the prepared sections from untreated tissue. This observation suggested many possibilities.

It was recalled that Kostoff³ working with amphidiploids stated that he had employed various chemical substances in treating plant tissue. His work and similar studies by others had been reviewed by the writer in a thesis⁴ submitted several years previously. With these investigations as a background and an active interest in a polyploid series of plants, the Resedaceae,⁵ it was natural for the writer to have an interest in any substance which might seem to offer the possibility of affecting the mitotic process. A small quantity of colchicine was obtained and a series of more or less successful preliminary experiments with plant tissues was begun.

Of course, onion root tips, radish and corn seedlings, with large nuclei and readily stainable chromatin offered distinct advantages for this type of investigation, so that most of the preliminary work up to the end of April, when the writer left Cold Spring Harbor, was done with these materials.

As the work progressed the main objectives of this initial cytological study using colchicine became centered around the following points: (1) the effect of colchicine upon individual embryonic cells rather than entire tissues; (2) the degree of polyploidy in a cytologically changed nucleus and the relation of the change to the method of treatment; (3) observable cytological changes due to the effects of colchicine upon the mitotic process; (4) the probable effectiveness of colchicine as a means of producing hereditary changes.

Since the cell reacts to the colchicine environment, independent of adjacent cells, it must be studied as an independent functional and structural unit. Therefore it is vital to consider the cytological effects upon individual embryonic cells of stem and root meristems.

Stem and root meristems of germinated *Zea mays*, *Raphanus sativa* and bulbs of *Allium cepa* were used to study the effect of colchicine upon their embryonic cells. The production of cytogenetical changes was found to be dependent upon three factors of the treatment, namely: (a) the concentration of colchicine solution, (b) the time allowed for the solution to act upon the meristem and (c) the physiological activity of embryonic cells at the time of treatment.

It is well known that embryonic cells are characterized by their ability to undergo mitosis and produce new cells. When meristematic cells divide mitotically the chromosomes organized from chromatin undergo equational longitudinal division into daughter chromosomes; these daughter chromosomes are completely separated from each other in metaphase, migrate to opposite poles in anaphase and subsequently reorganize as daughter

nuclei. It is also well known that the mitotic spindle formed during the process functions in the separation of daughter chromosomes and plays an important rôle in the formation of a cell plate between the daughter nuclei. The cell plate marks the place where the new cell wall is laid down to separate the two daughter cells and thus completes the mitotic process. The two new cells are genetically similar to each other and to the original parent cell. Interference or interruption of any one phase or several phases of the mitotic process could bring about the production of cells that differ from each other or from the parent cell. The colchicine treatments interrupted or inhibited certain phases of the mitotic process and without apparently affecting certain others.

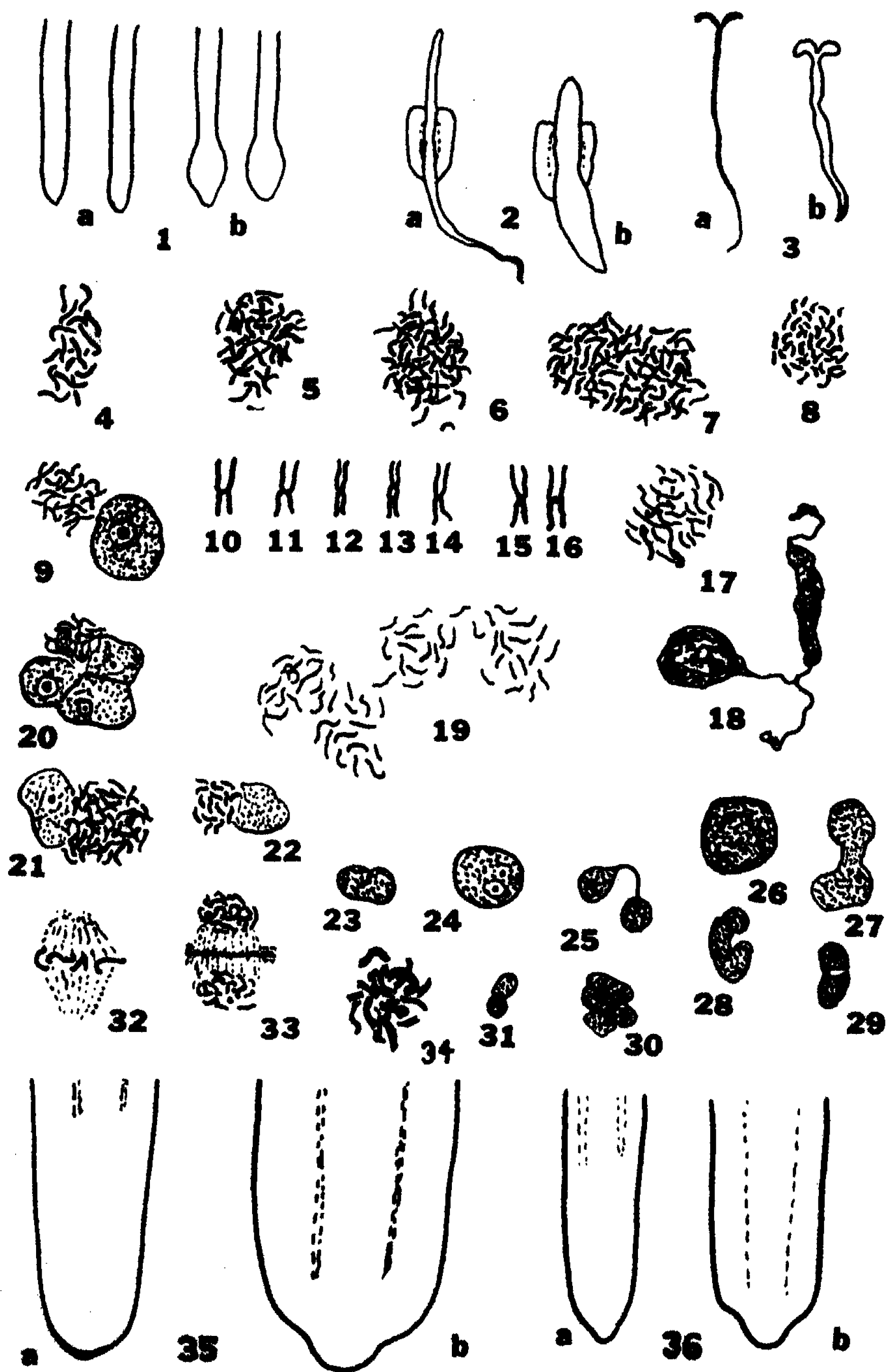
Colchicine is soluble in water and effective in very dilute solutions. The solutions used in these original studies were in concentrations of 1%, 0.1% and 0.01% and applied for 24, 48, 72, 96 and 108 hours. The seeds were germinated upon moist filter paper soaked with the desired concentration of colchicine for a given interval of time. *Allium* bulb root tips were treated by immersion. Definite toxic effects were observed from the highest concentrations. An increase in colchicine concentration or an increase in length of time for treatment beyond 72 hours was found to be detrimental and fatal to the future development of the meristematic tissue. There was a close correlation between the production of morphological abnormalities and the intensity and duration of the treatment. Increase in concentrations and increase in time for treatment caused increases in both structural abnormalities and cytological abnormalities.

Stem and root meristems were analyzed for cytological changes by two methods, namely, the aceto-carmin smear method, and the paraffin method for preparation of permanent slides. A weak Flemming solution was used for fixation and several different cytological stains were used for staining the sections. The principal plants used in this study were *Allium cepa*, *Raphanus sativa*, *Zea mays* and *Triticum vulgare*. The general morphological and tissue changes which appeared in these stem and root meristems were all similar in character. Since *Allium* root meristems offered material with large nuclei which were of advantage for cytological study this form was used most extensively for the detailed cytological studies given here.

A 0.1% and 0.01% colchicine solution both produced enlargements of *Allium* root, and stem portions, figures 1b, 2b, 3b, were consistently larger than untreated material, figures 1a, 2a, 3a. A decrease in the concentration of colchicine solution increases the length of time necessary for production of the enlargement. A fairly concentrated solution of 1% has

EXPLANATION FOR FIGURES

Figure 1.—Treated and untreated *Allium*. Figure 2.—*Zea mays*. Figure 3.—*Raphanus sativa*. Figures 8, 22, 31 and 36, *Zea mays*. Other figures *Allium*. Cytological figures Mgn. $\times 650$.



only a toxic effect, it does not produce enlargement even though the treatment is applied for sufficient time to produce change. The first sign of enlargement was found in the region of elongation some distance behind the dividing embryonic cell region. As the enlargement increased and time of treatment was prolonged, the bulbous portion came closer to the tip of the root.

This observation of enlargement over several days showed that as the swelling continued it approached the very tip of the root, and a similar progression of vascular elements were differentiating much nearer to the tip in treated roots, figures 35b, 36b, than in untreated root tips, figures 35a, 36a.

A section through one of these roots, figures 35b, 36b, shows this same progression in the differentiation of the vascular elements as compared with the untreated root tips, figures 35a, 36a.

The treatment is specific for individual cells, producing one type of change in a particular cell while the neighboring cell may be affected differently. This cytological variation from cell to cell was a characteristic feature. Such phenomena afford a basis for sectoral mutation as root tips and stem tips differentiate from the growing point.

The relationship of individual cellular reaction to the effects of colchicine, independent of adjacent cell activity is correlated with our knowledge of the individuality of mitotic activity found restricted to the activity of the cell and not the entire tissue. The enlargement of the tissue as a member is caused by increase in cell size rather than increase in cell numbers.

The chromosomes undergo longitudinal equational division, shown by a study of isolated chromosomes in aceto-carmin smear preparations, figures 10-16, which were made from colchicine treated root meristems of *Allium*. Colchicine does not inhibit or interfere with this process of chromosome division. The separation of the chromosome halves was complete except for a proportion of the achromatic region which seems to be associated with the constrictions of chromosomes. This phase of the mitotic process (longitudinal equational division of the chromosomes) must occur to produce polyploid nuclei, and during or after a moderate colchicine treatment this process continues without interruption.

The mitotic spindle or the formation of an achromatic figure was definitely inhibited in colchicine treated material as shown by chromosomes plates in aceto-carmin preparations of *Allium* in figures 5, 6, 7, 17 and 19. A paraffin section which was specifically prepared with weak Flemming solution, figure 34, and stained, with the triple stain, failed to show spindle formation. Similar material untreated and fixed in Flemming solution, and similarly stained, showed the presence of the spindle and chromosomes in a definite equatorial plate, figure 32, and a later mitotic

stage, figure 33, showed development of the spindle and cell plate between nuclei. From this and similar observations it was concluded that colchicine prevents spindle formation.

The diploid number of chromosomes for *Allium* is 16, shown in figure 4 from an untreated control. Counts of chromosome numbers in *Allium* after 48 hours of treatment with colchicine were 32 in figure 5; 48 in figure 6; 64 in figure 7. For the treated *Zea mays* stem meristems, figure 8, 40 chromosomes were counted. Application of pressure to the cover slips of aceto-carmin preparations made it possible to count the higher numbers. The evident cytological change was one of polyploidy. Polyploid cells could be found in both stem figures 8, 22, and root meristems, figures 5, 7, following colchicine treatment. Polyploidy of cells in the meristems of stems as well as root tissue indicates that colchicine may be valuable for the induction of polyploidy with practical significance, because these cytogenetical variations produced in the stem and transmitted by growth to the reproductive organs could be used for purposes of propagation and plant breeding.

Up to April the writer's investigation had not progressed beyond the cytological observations given in the paper, but it was felt that heretofore it had not been possible to induce cytogenetic changes by the application of reagents to embryonic cells in such an effective manner as was indicated by these preliminary studies. Researches by Nemec,⁶ Winkler,⁷ Jorgensen,⁸ Randolph⁹ and Kostoff,³ had also shown that there were possibilities of inducing cytogenetic changes in various ways.

Blakeslee,^{10,11} who made reference to the writers' unpublished results, and encouraged this publication has since proved beyond doubt that in *Datura*, tetraploids may be obtained from diploids after colchicine treatment. Likewise, Nebel¹² has been carrying out a series of cytological studies on both plant and animal tissue which indicated the same possibility. Thus it appears likely that the use of colchicine will prove to be of great practical importance to genetics.

Multinucleate structures were produced in *Allium* after colchicine treatment as shown in figures 9, 20, 21, 23, 25, 27, 28, 29, 30 and in *Zea mays* stem meristematic cells, figure 31. These conditions are probably due to prolonged treatment in solutions that were not entirely toxic. Each part of the multinucleate structure or compound nucleus represents the diploid number, the chromosomes of which are capable of dividing to produce an octoploid nucleus, figure 19. In this way octoploid nuclei are derived from tetraploid nuclei. It is possible for one part of the compound nucleus to divide, figure 9, while the other part remains in interphase. The result of this process would be a 48-chromosome or hexaploid nucleus, figure 6. The fact that polyploid nuclei have formed during treatment and that with prolonged treatment these polyploid nuclei again divided to form hexaploids

or octoploids, indicates the reason why colchicine is effective in polyploid induction. It is because this reagent does not inhibit chromosome division, but does prevent spindle and cell plate formation. The multinucleated structures are abnormal cytological monstrosities but may be regarded as components of polyploid nuclei in cells, some of which may still be capable of dividing later to form tissue. It is possible that this is the answer to the question regarding the peculiar effectiveness of colchicine as an agent.

The binucleate condition was observed in stem meristems of treated *Zea* tissue, figure 31. Another cell in *Zea* tissue showed that a portion of the nucleated component is capable of forming additional chromosomes, figure 22. The presence of conditions in stem meristems similar to root meristems indicated that principles of polyploid induction found to be true for roots were also true for stems.

The multinucleate portions adhere to each other so that separation of the compound nucleus from the cell does not break up the nucleus into its component nuclear parts. In many cells the polyploid condition is present in the form of a single nucleus. In these cases the existence of multiple numbers of chromosomes could be inferred by comparison of size of nucleus.

Induction of polyploidy by colchicine occurs in two general stages: namely (1) a stage marked by increased chromosome number without nuclear abnormalities and irregularities, and (2) the induction of polyploidy with abnormal shapes of nuclei and abnormal counts. The latter class is characteristic of cells treated for a long time and with greater concentrations of colchicine. These results were found in some cases recorded with abnormal nuclei, figures 18, 25, 27 and abnormal dividing chromosomes, figure 17. The former class marked by polyploidy without abnormalities was a group of changes produced by treatment for shorter periods of time and less concentrated solutions. It is that class of induced polyploidy which is capable of division during treatment and after treatment in which we are particularly interested, from the point of view of hereditary problems. The abnormal nuclei, figure 18, were only cytological monstrosities and more characteristic for the conditions produced by root tip treatments with chloral hydrate and other chemicals, or external environmental agents. Abnormal phases showed more fusions of chromosomes, fragments lost from nuclei and other more unusual cytological occurrences.

The physiological activity of the embryonic cell at the time of treatment is important because increase of cell activity means increase in number of mitotic divisions. This increase in mitotic division renders colchicine treatment more effective. The methods used in this study with treated seedlings already germinated and growing meristems will not yield precisely the same results when applied to the relatively inactive meristematic cells of dry seeds.

This study demonstrated that colchicine is effective in the production of cytogenetically changed cells. The process affects the mitotic divisions; hence, it is a study of independent cells as structural and functional units. These units divide mitotically at a given time and do so independently of the activity of the adjacent cells. The writer was not concerned with a study of entire tissues influenced by colchicine treatment. The exact rôle played by colchicine is essentially the inhibition of the mitotic spindle which prevents separation of the daughter nuclei, and cell plate formation, with the subsequent division into two cells. The failure of the reagent to interfere with the process of chromosome formation by longitudinal equational divisions, shows a specificity of a high degree for inhibition of certain phases of cell division and the apparent promotion of other phases of the mitotic process.

¹ Allen, E., *Anat. Rec. Abst.*, 67, 49 (1936).

² Mr. E. L. Lahr gave the writer some helpful suggestions in the initiation of this investigation.

³ Kostoff, D., *Genetica*, 12, 33-118 (1930).

⁴ Eigsti, O. J., *Manuscript Thesis*, University of Illinois (1935).

⁵ Eigsti, O. J., *Bot. Gaz.*, 98, 363-369 (1936).

⁶ Nemec, B., *Jahr. Wis. Bot.*, 89, 645-730 (1904).

⁷ Winkler, H., *Zeits. Bot.*, 8, 417-531 (1916).

⁸ Jorgenson, C. A., *Jour. Genet.*, 19, 133-211 (1928).

⁹ Randolph, L. F., these PROCEEDINGS, 18, 222-229 (1932).

¹⁰ Bakeslee, A. F., *Compt. Rend.*, 205, 476 (1937).

¹¹ Blakeslee, A. F., *Jour. Hered.*, 28, 392-411 (1937).

¹² Nebel, B. R., *Biol. Bull.*, 73, 351-352 (1937).

CHEMICAL STRATIFICATION AND LAKE MORPHOLOGY

BY G. EVELYN HUTCHINSON

OSBORN ZOÖLOGICAL LABORATORY, YALE UNIVERSITY

Communicated January 11, 1938

Data collected during an extended investigation of Linsley Pond, a small eutropic lake (area 94,400 m.², max. depth 14.8 m., mean depth 6.7 m.) near North Branford, Connecticut, throw considerable light on the nature of the water-movements in the hypolimnia of thermally stratified lakes. That such movements occur is clear from the rise in concentration of substances, that can only have been derived from the bottom mud, at distances from the latter far exceeding those over which molecular diffusion could be effective. The nature of such water movements has been a matter of discussion, three main hypotheses having been advanced in recent years. Birge,¹ Thienemann² and more explicitly Grote,³ have regarded

wind-generated turbulence as the most important agency in the transport of heat and dissolved material throughout the lake. This hypothesis implies that the hypolimnion is not really a closed system and that considerable vertical turbulent diffusion occurs. McEwen,⁴ while admitting the fundamental importance of turbulence, believes that at least in the upper hypolimnion and in the epilimnion, a system of vertical convection currents generated by surface cooling, is of great importance. Alsterberg,⁵ on the other hand, regards the hypolimnion as a closed system, in which water movements are confined to thin horizontal laminae, such movements having a negligible vertical component. This hypothesis appears to be the only one consistent with the remarkable optical microstratification discovered by Whitney.⁶ It is perhaps unfortunate that in previous investigations most attention has been focused on temperature, in part controlled by direct radiation, and on oxygen, the most reactive of all dissolved substances in a lake, and in the present case known to be primarily controlled (Riley *unpublished*) by consumption and production in the free water. Evidence based on the distribution of alkalinity (bicarbonate ion, determined by titration with methyl orange as indicator) is given below in support of Alsterberg's hypothesis, though the results obtained on the oxygen deficit in Linsley Pond do not permit us to follow this author in all his conclusions.

Exclusion of Vertical Turbulent Transport.—In figure 1, the solid lines represent the variation, with depth, of alkalinity, the curves being based on the means of determinations made weekly, at one meter intervals, during three successive five-week periods, throughout the summer of 1937. It will be observed that during the period of investigation alkalinity tends to rise, but that the rise is much greater in the lower hypolimnion than in the epilimnion. Mineral analyses made at the end of September indicate that in the epilimnion sufficient calcium and magnesium exists to balance the bicarbonate, but that the concentration of these substances hardly varies with depth. In the hypolimnion, the excess bicarbonate is apparently balanced by considerable quantities of ammonia and iron, the latter presumably in the ferrous condition. This iron can only have come from the bottom and the large amounts of ammonia appear to indicate a like origin for the volatile alkali.

At any depth (y), the rate of change $\frac{\partial \theta}{\partial t}$ in any property (θ) with time due to turbulent diffusion is given by

$$\frac{\partial \theta}{\partial t} = \mu^2 \frac{\partial^2 \theta}{\partial y^2},$$

where (μ^2) is a virtual diffusion coefficient, the coefficient of turbulence, which is always positive. If the second derivative with respect to depth be negative, the first derivative with respect to time must be negative also.

If the three curves be compared, it appears that they show a marked similarity in form, showing points of inflection at the same depths. (Cf. also figure 2.) These points of inflection are such that in the lower water,

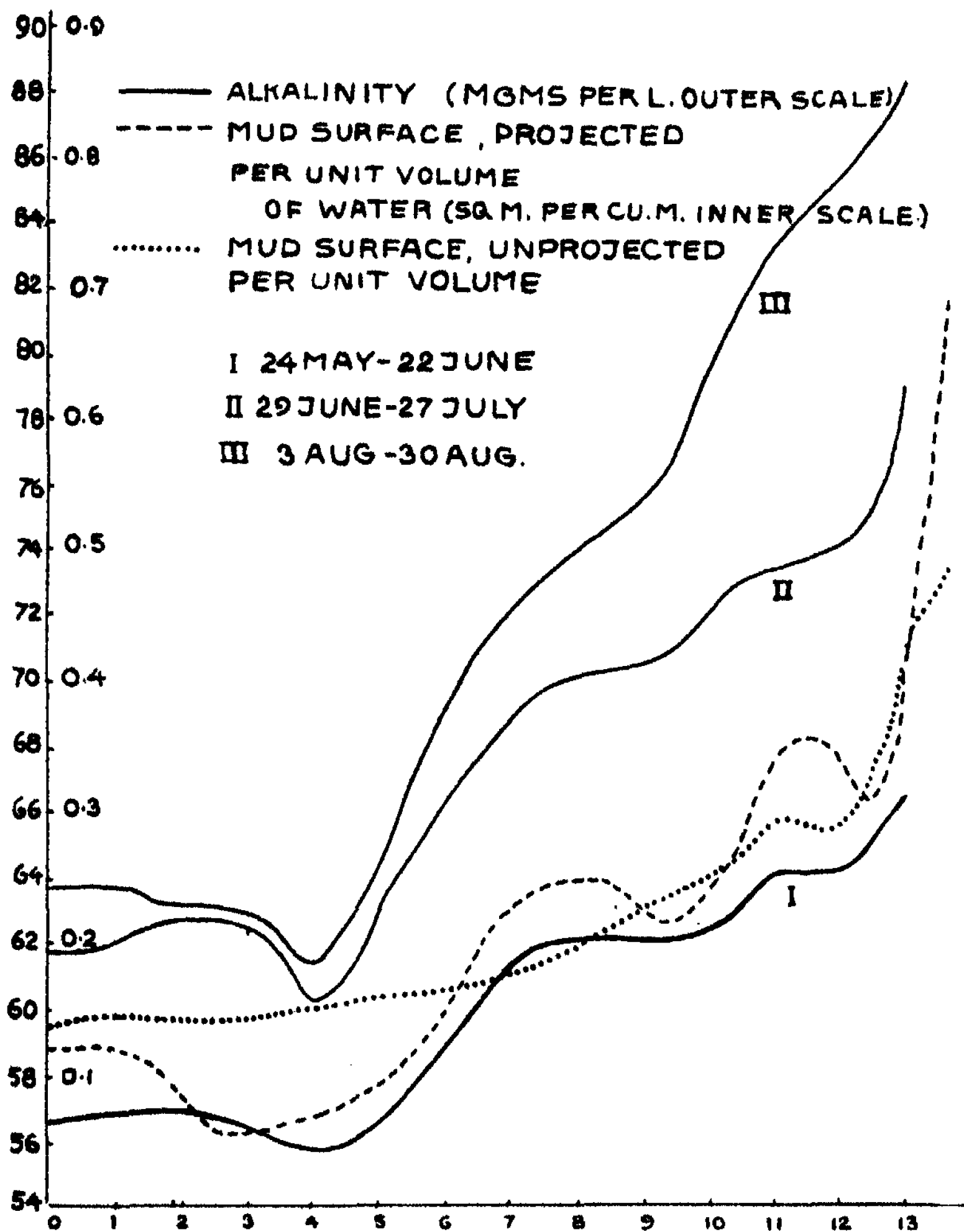


FIGURE 1

Solid lines, variation of alkalinity (HCO_3) with depth (mean values over successive five week periods). Broken line, ratio of projection of mud surface to volume. Dotted line, ratio of mud surface to volume.

the second derivative with respect to depth is persistently negative at 8 m. and 11 m. If the rise in alkalinity were due to turbulent movement bringing ferrous and ammonium bicarbonate up from a constantly re-

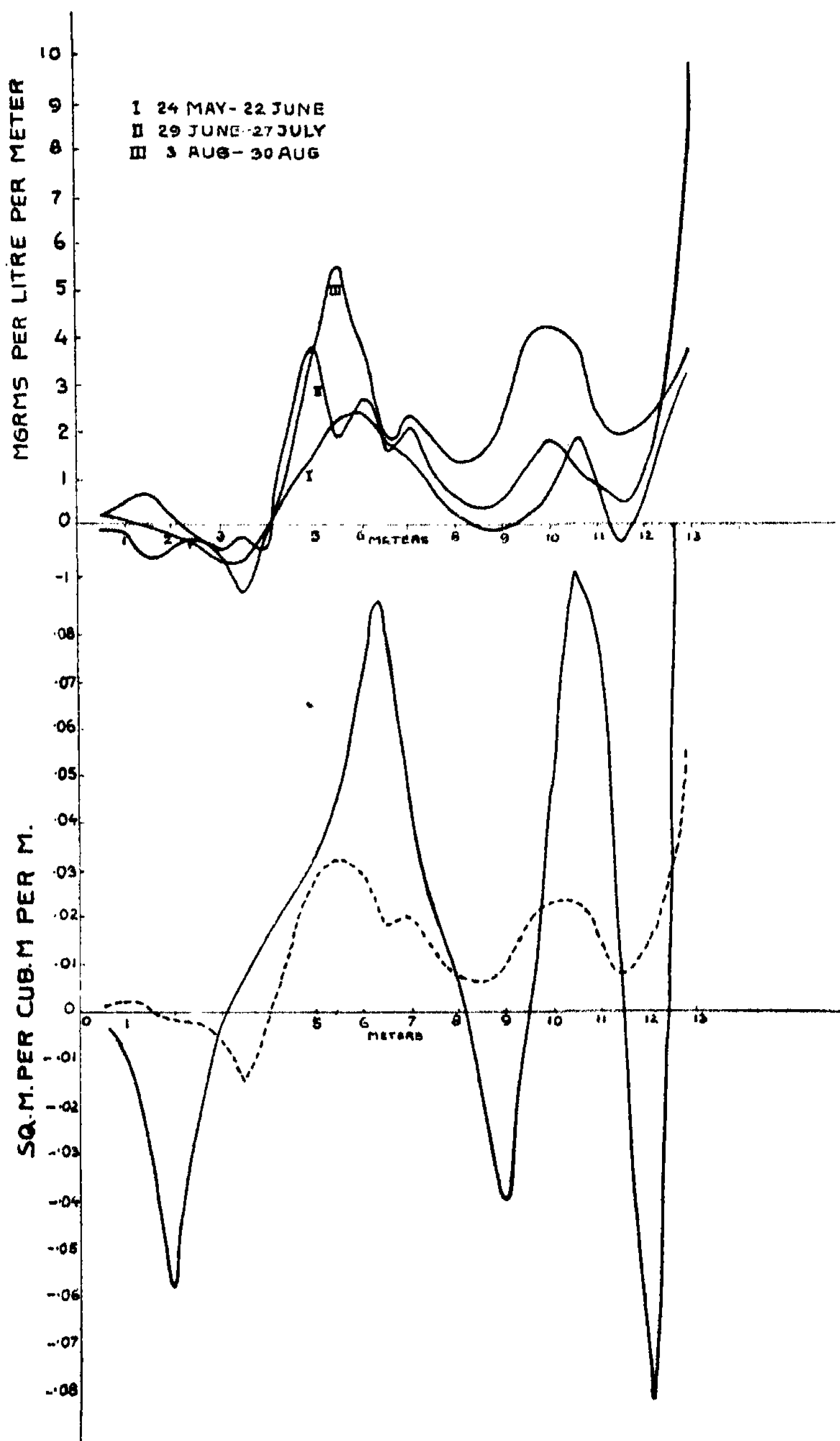


FIGURE 2

Upper curves, first derivative of alkalinity curves in figure 1. Lower curve, solid, first derivative of projected mud surface curve; broken, mean of the three upper curves.

generated and highly concentrated microzone on the mud, it is clear that the persistence of sections of the curve with a negative second derivative would be quite inconsistent with the continued accumulation of bicarbonate at all levels of the hypolimnion as a whole. A similar situation is apparent in the individual curves relating alkalinity to depth during periods of rapid rise in alkalinity. Such curves are less regular than the mean curves of figure 1, doubtless owing to uneven horizontal distribution at any given time, but regions with a negative second derivative can always be found, though not invariably at the same depths as in the smoothed curves. The unaveraged data, therefore, likewise give ample evidence of the occurrence of considerable rises in alkalinity in layers of water where the turbulence hypothesis would, as in the case of the average curves, indicate that the alkalinity should be decreasing.

Particular significance is to be attached to the occurrence of a zone with a negative second derivative at 11 m., because, if McEwen's theory be accepted, this region should show the effects of almost pure turbulence uncomplicated by the type of convection current that this author has postulated. While it is hoped in the future to examine the implications of this very complex theory in greater detail, the correlation indicated below strongly suggests that it is inapplicable in the present case.

Morphological Concomitants of Horizontal Streaming.—As Alsterberg himself pointed out, if the hypolimnion be regarded as closed, and if its water movements are practically exclusively horizontal, a relationship should exist between the chemical characters of any layer of free water, and the amount of mud surface to which the edges of the layer are exposed. In attempting such a correlation it must be remembered that a steeply sloping lake bottom will probably neither receive nor retain as much mud as one that is more horizontal. A practical investigation of this problem would be extremely difficult, but as a first approximation it has been assumed that the chemically active mud is proportional not to the actual area of the lake bottom, but to this area projected on to a horizontal plane.

The broken line in figure 1 represents a curve drawn by plotting at 0.5 m., 1.5 m. and at succeeding meter intervals, the ratio of the area between successive one meter contours on a bathymetric map of the lake, to the volume enclosed between the planes represented by these contours. A comparison of this curve with the alkalinity curves of the same figure indicates that a relationship of the type demanded by Alsterberg's horizontal current hypotheses does exist. This is further brought out when the derived curves in figure 2 are examined. Owing to the large amount of steep lake bottom, particularly on the western side of the basin, the relationship is obscured when the ratio of contour length to area is taken as the measure of mud to water at any depth (dotted line in figure 1). The existence of any such relationship, even though it involve an empirical

but quite objective correction, speaks very strongly in favor of the hypothesis of horizontal movement, and this, taken in conjunction with the

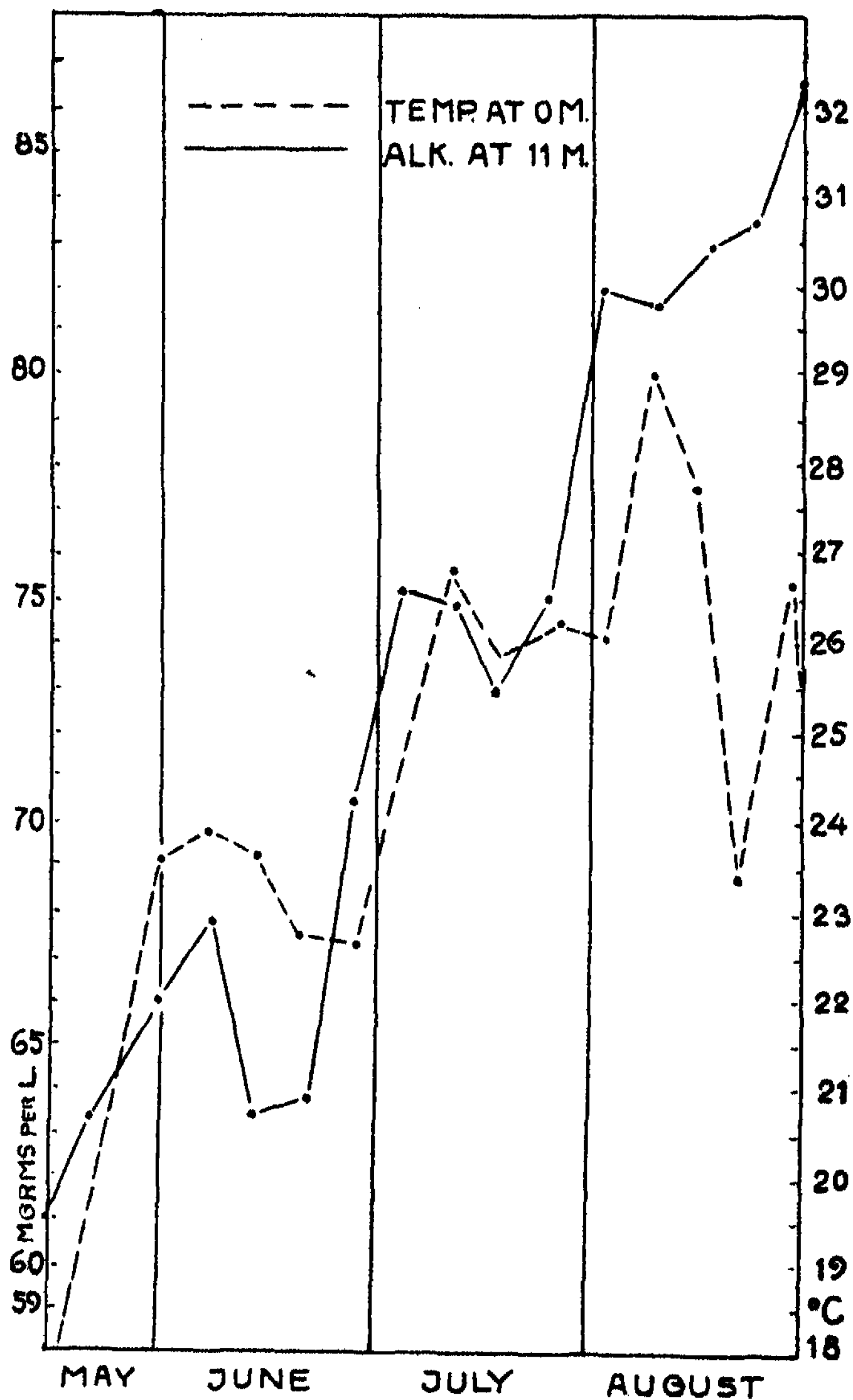


FIGURE 8

Variation of alkalinity at 11 m. (solid), and of temperature at 0 m. (broken), with time.

evidence given above, makes any other hypothesis of hypolimnetic water movements most improbable.

Temporal Variation in Alkalinity.—Conclusions based on comparison of the details of curves showing the variation of alkalinity with time at any given depth, are, for various technical and other reasons, less satisfactory than those based on the depth distribution curves for any given day. Although the significance of certain features in such curves is doubtful, the solid line in figure 3, representing the alkalinity at 11 m. from the end of May to the end of August, and typical of the events of all depths, indicates marked discontinuities in the rise of alkalinity during this period. The cause of the minima before the sudden rises is at present obscure, the two large rises (22 June–6 July and 27 July–3 August) certainly lie outside experimental error, and since the high values reached are in each case maintained almost unchanged for a week, they must be regarded as real and general throughout the water layer. These two large rises, and a third discontinuous rise beginning on the 24 August, appear to be initiated by temperature minima *at the surface* (broken line). Examination of the density curves at the time of such minima indicated that the stability of the top two meters of the lake is reduced at such times and rises more suddenly between two and three meters than at times of temperature maxima. This suggests that though the rise in alkalinity cannot be due to vertical turbulent diffusion, the best conditions for such a rise are provided by the existence of a well defined and freely circulating epilimnion of measurable thickness below which the stability rises sharply. Preliminary experiments with small scale models indicate that horizontal streaming of the type postulated can be produced in a lower, more dense layer when a less dense layer of water lies upon it, and when the boundary between the two is made to oscillate by an air current which causes turbulent motion in the upper layer, but no mixing of the upper, less dense water with the denser water at the bottom of the tank. It is hoped to be able to investigate the nature of such currents in models under more favorable conditions in the near future.

Intensive work in 1937 was made possible by a grant from the BACHE FUND, of the NATIONAL ACADEMY OF SCIENCES, here gratefully acknowledged. My thanks are also due to Professor A. E. Parr, for the loan of apparatus, to Mr. H. J. Turner and Miss Anne Wollack for the care they have taken over analyses entrusted to them, to Dr. G. A. Riley for unpublished morphometric data and to Dr. Riley and Mr. E. S. Deevey for help in the field.

¹ Bfge, E. A., *Trans. Wisc. Acad. Sci. Arts. Lett.*, 18, 341 (1916).

² Thienemann, A., *Die Binnengewässer*, 4, (1928).

³ Grote, A., *Die Binnengewässer*, 14, *Arch. Hydrobiol.*, 29 (3), 410 (1935).

⁴ McEwen, G. F., *Bull. Scripps. Instn. Oceanogr. Tech.*, 2, 197 (1929).

⁵ Alsterberg, G., *Bot. Notiser*, 255 (1927).

⁶ Whitney, L. V., *Science*, 85, 224 (1937).

ASCORBIC ACID AND THE GROWTH OF PLANT EMBRYOS

BY JAMES BONNER AND DAVID BONNER

WILLIAM G. KERCKHOFF LABORATORIES, CALIFORNIA INSTITUTE TECHNOLOGY

Communicated December 27, 1937

Introduction.—In an earlier paper it has been shown¹ that excised pea embryos of the variety "Perfection" may be satisfactorily grown upon sterile nutrient medium, particularly if various "accessory growth factors" of known chemical composition are provided. It was shown that there are at least four of these growth factors, one of which is ascorbic acid. It is ascorbic acid with which the present communication is particularly concerned.

There have been, previously, conflicting reports as to the effect of ascorbic acid upon plant growth. Havas,² von Hausen³ and Davis, Atkins and Hudson,⁴ among others, have found that ascorbic acid may exert a beneficial effect upon the growth of certain plants. It is of particular importance in the present connection, however, that Kögl and Haagen-Smit,⁵ who grew pea embryos *in vitro* under conditions similar to those used by the present authors, failed to find any growth factor activity for ascorbic acid. They worked with pea varieties other than "Perfection," and a varietal difference in the ability of pea embryos to cover their ascorbic acid requirements by synthesis might therefore be suspected. It will be shown below that the embryos of different pea varieties do indeed differ widely in their ability to synthesize ascorbic acid from sucrose. It will in addition be shown that those varieties which synthesize the smallest amounts of this substance are those which respond (with increased growth) to its addition to the culture medium. The varieties which synthesize relatively large amounts of ascorbic acid, on the other hand, apparently furnish themselves with sufficient amounts of the substance and do not respond to its addition to the external medium.

Methods.—The nutrient medium used for the culture of the excised embryos was that used in the previous experiments,¹ which contained, per liter, the following constituents: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 236 mgs.— $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 36 mgs.— KNO_3 , 81 mgs.— KCl , 65 mgs.— KH_2PO_4 , 12 mgs.—ferric tartrate, 1.5 mgs.—sucrose, 40 gms. and agar, 10 gms. Fifteen cc. of this medium was placed in each of the 50 cc. Erlenmeyer flasks which were used as the culture vessels. 0.75 Mgs. of ascorbic acid was added to each culture flask in the ascorbic acid cultures. The pea seeds were sterilized in 95% alcohol and 0.1% HgCl_2 , and were then soaked in sterile water for six hours prior to excision of the embryos. The length of time for which the seeds are soaked is of considerable importance, since with times longer than six hours, increasing amounts of the various accessory growth factors are ap-

parently mobilized from the cotyledons by the embryo. Twenty embryos were used for each treatment or variety in each experiment, and each experiment consisted of 10 parallel portions, or a total of 200 embryos. The actual culturing was carried out in a special culture room, after which the embryos were grown in a dark room thermostatically controlled at 24 C. Ascorbic acid determinations were made by extraction of the tissues with 2% meta-phosphoric acid in 8% acetic acid according to the method of Muslin and King,⁶ and subsequent titration of the extract with a standard 2-6-dichlorophenolindophenol solution in a micro-burette.

Growth measurements of the seedling shoots were made weekly for four weeks from the time of excision of the embryos, and the measurements from

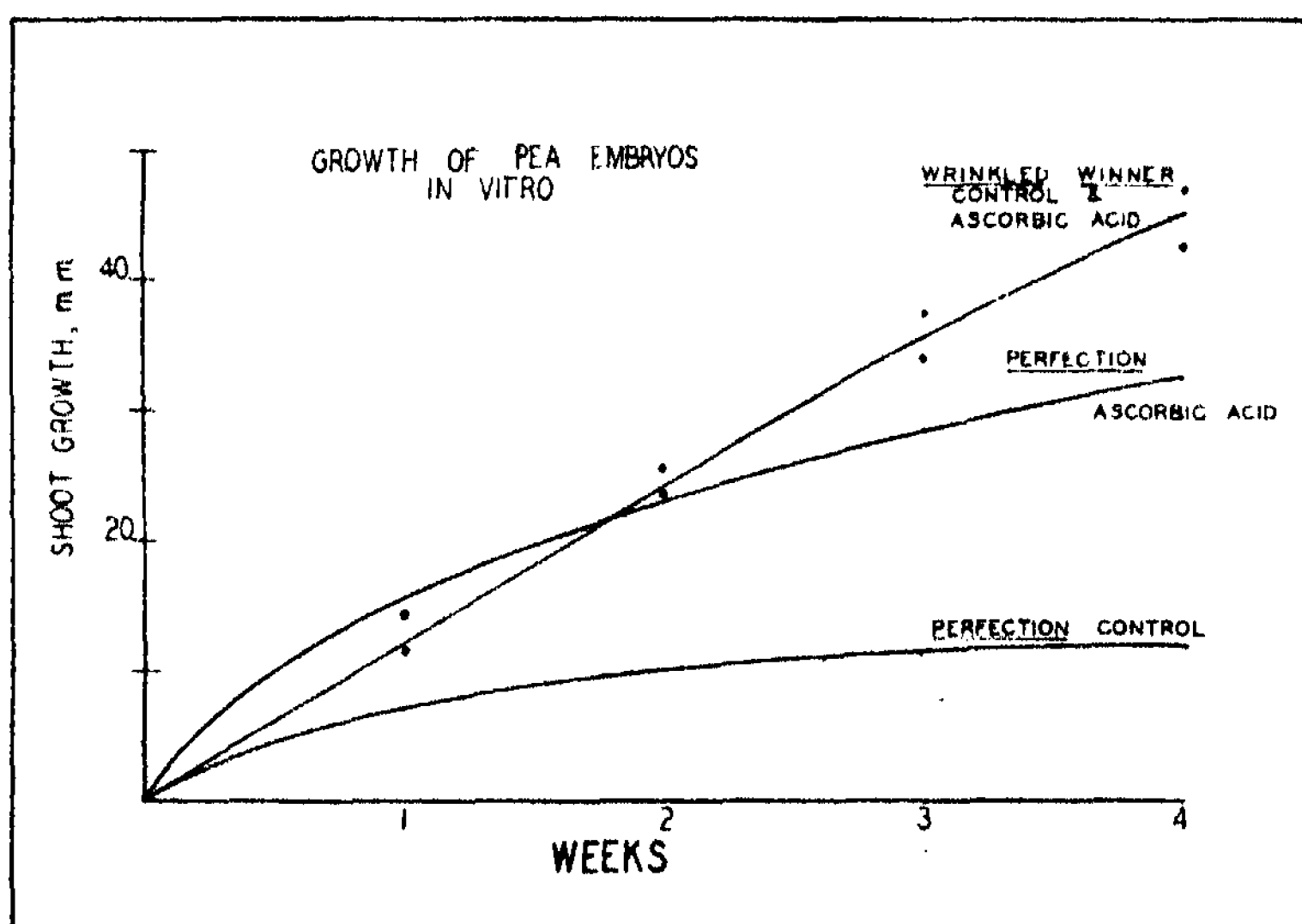


FIGURE 1

The growth rate of pea embryos *in vitro*, the varieties "Perfection" and "Wrinkled Winner," each with and without the addition of ascorbic acid to the nutrient medium.

a typical experiment are presented in figure 1. Preliminary experiments also showed that the ascorbic acid content rises to a maximum within two weeks and that it remains at approximately this maximum level for the following two weeks. The ascorbic acid determinations were therefore made only on the plants whose growth had been followed for four weeks. This had of course the advantage that the growth measurements and the ascorbic acid determinations were made upon the same individual plants. In the discussion below only the shoots will be considered. It was, however, found that there is a close correlation between the ascorbic acid content of root and shoot.

Experimental Results.—Nine varieties of pea embryos were compared as to synthesis of and growth response to ascorbic acid.⁷ The length of shoot, response to ascorbic acid and content of ascorbic acid, all at the expiration of four weeks, are summarized for each of these nine varieties in table 1 and presented graphically for two typical varieties in figure 2. It may be seen at once that the varieties fall sharply into two classes, i.e., those which respond to added ascorbic acid with greatly increased growth, and those which show little or no growth response. Of the latter the variety

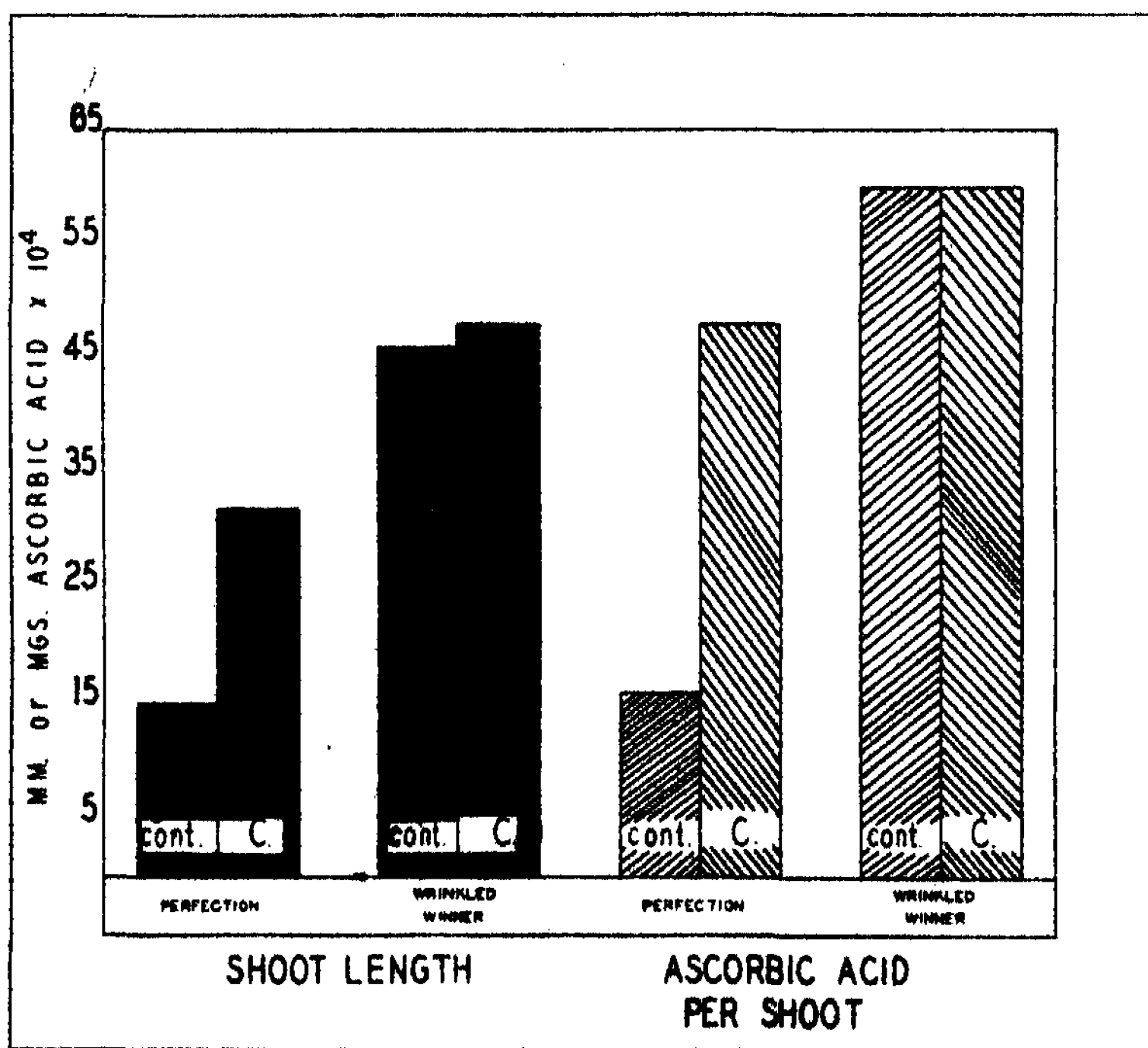


FIGURE 2

The growth and the ascorbic acid content of pea embryos, varieties "Perfection" and "Wrinkled Winner" after four weeks *in vitro*. "cont." = control cultures, without added ascorbic acid. "C." = cultures receiving ascorbic acid.

"Wrinkled Winner" is the best example, since the four other varieties proved more difficult to grow *in vitro* and gave rather irregular results. Correlated with growth response to added ascorbic acid is *low ascorbic acid content* of the variety when it is grown on the vitamin free basic medium. The varieties "Perfection" and "Alaska" which respond vigorously contain less than one-third as much ascorbic acid as embryos of "Wrinkled Winner" which does not respond. Neither "Perfection" nor "Wrinkled

Winner" embryos contain appreciable amounts of ascorbic acid when they are excised from the seed at the end of six hours' soaking. The above differences of ascorbic acid content of the seedlings must then be due to *differences in the ability of the various varieties to synthesize ascorbic acid*. We see at once that the fact that some varieties of pea embryos do not respond to ascorbic acid does not by any means justify the conclusion that such embryos do not need this substance. It would rather seem to indicate that such embryos are capable of synthesis of ascorbic acid in quantities sufficient to fulfill their requirements.

Plants which are supplied with ascorbic acid actually take it up and their content of this substance is correspondingly increased above that of control plants, as is also shown in table 1 and graphically in figure 2. The ascorbic acid content of the variety "Perfection" when supplied with ascorbic acid, is not greatly below that of the variety "Wrinkled Winner" which synthesizes the substance in adequate amounts. Thus, when "Perfection" embryos are supplied with ascorbic acid, their vitamin content as well as their growth rate increases to a level comparable to that of the ascorbic acid "auto-trophic" variety, "Wrinkled Winner."

TABLE 1
ASCORBIC ACID CONTENT AND GROWTH RESPONSES OF NINE VARIETIES OF PEA EMBRYOS *in Vitro*

VARIETY	TOTAL PLANTS USED	AVERAGE GROWTH IN 4 WEEKS, MM.			MOB. ASCORBIC ACID PER;		
		CONTROL	ASCORBIC ACID	(% OF CONTROL)	CONTROL SHOOT	ASCORBIC SHOOT	GM. WET WEIGHT, (CONTROL)
Perfection	100	15	32	213	0.0016	0.0048	0.064
Alaska	40	23	46	200	0.0016	0.0035	0.075
Daisy	40	10	17	170	0.0015	0.050
British Empire	40	11	18	163	0.0012	0.054
Delacatessa	20	29	33	114	0.004	0.138
Little Marvel	20	37	38	103	0.006	0.120
Laxtons Progress	20	24	25	104	0.006	0.167
Stratagem	20	22	23	104	0.005	0.145
Wrinkled Winner	100	46	48	104	0.006	0.006	0.172

The control embryos of the five varieties which synthesize relatively large amounts of ascorbic acid also grow somewhat better than the control embryos of the four varieties which are ascorbic acid "hetero-trophic," although the variety "Alaska" is apparently an exception. It seems nevertheless that in general, ability to synthesize ascorbic acid is correlated not only with a lack of response to added vitamin, but also with a larger absolute growth in the absence of added vitamin.

As mentioned above, the excised pea embryo is able to respond with increased growth to several different accessory factors, even though one of these factors be added to the medium in the absence of the others. Thus, aneurin (vitamin B₁) alone acts as an accessory growth factor just as does

ascorbic acid.¹ However, the addition of aneurin to the medium considerably increases the amount of ascorbic acid present in the plant, as is

TABLE 2
INFLUENCE OF ANEURIN (VITAMIN B₁) ON THE ASCORBIC ACID CONTENT OF
"PERFECTION" PEA EMBRYOS

	GROWTH IN 4 WEEKS; MM.	ASCORBIC ACID CONTENT; MGS. PER SHOOT
Control Embryos	15	0.0018
Aneurin Embryos	40	0.0052

shown in table 2. In this typical experiment there was found to be nearly three times as much ascorbic acid in the plants which received aneurin (0.01 mgs. per culture flask⁸) as in those which did not. The nature of this effect cannot as yet be explained, but it is of interest to note that Svirbely⁹ reports that synthesis of ascorbic acid by the rat depends upon an adequate supply of vitamin B₁.

Discussion.—The point which it is particularly desired to emphasize in the present paper is that an organism will or will not respond to a given accessory growth factor depending, among other things, on (a) the need of the organism for the factor in question, and (b) the ability of the organism to synthesize the substance. The fact that a given organism does not respond to a given growth factor may then mean only that the particular organism is equipped to synthesize it in adequate amounts; it does not necessarily mean, however, that the factor in question plays no rôle in the economy of the organism. This principle has long been familiar to the animal physiologists, who realized that the rat, for example, covers its ascorbic acid requirements by synthesis. The same principle has also been demonstrated for the yeasts by Lucas¹⁰ and others, and more recently for other of the fungi by Kögl and Fries.¹¹ It would seem important that this principle be also recognized in plant physiology, in order that controversies as to the "essential" or "non-essential" nature of the various growth factors be avoided. It has been shown above that plants as closely related as the various varieties of peas differ greatly as to their response to ascorbic acid. For varieties such as "Perfection" and "Alaska" ascorbic acid would seem, superficially, to be more nearly an essential growth factor than for varieties such as "Wrinkled Winner." In reality, however, these varieties differ rather in their ascorbic acid auto-trophism, in their ability to synthesize the substance, and it would seem justifiable to conclude that ascorbic acid is quite as much a growth factor for "Wrinkled Winner" as for "Perfection."¹²

¹ J. Bonner and Grice Axtman, *Proc. Nat. Acad. Sci.*, 23, 453 (1937).

² L. Havas, *Nature*, 136, 435 (1935).

³ S. von Hausen, *Biochem. Zeit.*, 288, 378 (1936).

⁴ W. Davis, G. Atkins and P. Hudson, *Ann. Bot. [N. S.]*, 1, 329 (1937).

⁴ F. Kögl and J. Haagen-Smit, *Zeit. Physiol. Chem.*, **243**, 209 (1936).

⁵ R. Muslin and G. King, *Jour. Biol. Chem.*, **116**, 409 (1936).

⁷ For seed of the various pea varieties the authors are greatly indebted to the Ferry-Morse Seed Co., San Francisco, and to the Calif. Packing Corp., San Francisco.

⁸ The authors are indebted to the Merck Co., Inc., Rahway, N. J., for the supply of synthetic aneurin.

⁹ J. Svirbely, *Amer. Jour. Physiol.*, **116**, 445 (1936).

¹⁰ G. Lucas, *Jour. Phys. Chem.*, **28**, 1180 (1924).

¹¹ F. Kögl and N. Fries, *Zeit. Physiol. Chem.*, **249**, 93 (1937).

¹² Published as a report on Work Project No. 6330-6989, Official Project No. 165-036999, conducted under the auspices of the Works Progress Administration. The authors are deeply indebted to Philip Divirian for his able and continuous assistance during the prosecution of this work.

POTENTIALS IN HALICYSTIS AS AFFECTED BY NON-ELECTROLYTES

BY W. J. V. OSTERHOUT

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated December 20, 1937

In diluting the sea water surrounding marine algae it is desirable to add something to maintain the osmotic pressure, otherwise the cells take up water and may burst. Damon¹ used glycerol for this purpose in experiments on *Valonia*. He found that the changes in P.D. at 20°C. obeyed the equation

$$\text{P.D.} = 58 \frac{V_{\text{Cl}} - U_{\text{Na}}}{V_{\text{Cl}} + U_{\text{Na}}} \log \frac{C_1}{C_2}$$

where V_{Cl} and U_{Na} are the apparent mobilities of Cl^- and Na^+ in the outer protoplasmic surface, C_1 is the higher and C_2 the lower concentration of sea water. Putting $C_{\text{Cl}} = 1$ he obtained 0.2 for the value of U_{Na} (on the assumption that $C_1 \div C_2$ was the same in the protoplasmic surface as in the external solution).

The limiting value of the P.D. in this case may be obtained by putting $U_{\text{Na}} = 0$. We then obtain 17.4 mv. as the limit when $C_1 \div C_2 = 2$. This value is a little less when we employ activities in place of concentrations.²

Similar experiments with *Halicystis*³ yield a different result. In view of the work of Blinks⁴ it was decided to add sufficient CaCl_2 and KCl to the glycerol solution to make the concentration approximately the same as in sea water. The solution contained 1.1 M glycerol + 0.02 M CaCl_2 + 0.012 M KCl .

When sea water is diluted with an equal volume of this solution (at pH 8.2) and is then substituted for natural sea water (at pH 8.2) we find that in

one minute⁵ or less the P.D. becomes about 29 mv. less positive.⁶ Since this value is in excess of the theoretical limit of 17.4 mv. it seems possible that glycerol has altered the value of $C_1 \div C_2$ in the protoplasmic surface so that instead of being 2, as assumed, it has reached some higher value.

This might happen as follows. If the partition coefficient S (conc. of electrolyte in protoplasmic surface \div conc. of electrolyte in external solution) were constant, the value of $C_1 \div C_2$ would be the same as in the external solutions. But if S in the dilute sea water is lessened by the glycerol, the value of C_2 will be correspondingly small and that of $C_1 \div C_2$ will be greater.

It seems advisable, for the present at least, to employ the term partition coefficient in a very broad sense since, for example, the effect of NaCl may depend not only on the number of sodium ions in the protoplasmic surface but also on complexes in the sense of Kraus,⁷ e.g. $(NaX_I)^+$ and $(NaX_{II})^+$ where X is an element or a radical. All such complexes in the protoplasmic surface plus the actual sodium ions may be lumped together and their sum in unit volume of the non-aqueous surface layer divided by the sum of the potassium ions in unit volume of the external solution may be called the "apparent partition coefficient."

A variety of other explanations is possible, such as the following:

(1) Mechanical rupture of the outer protoplasmic surface layer, the process being fully reversible. If this layer is liquid, as seems possible, this process might take place as in (unpublished) experiments with oily films spread out on the surface of aqueous solutions when chemical action is going on. In such cases breaks in the film may appear and disappear as the result of local action. A great many are usually present and the amount of short-circuiting and consequent loss of P.D. might change gradually, depending on the number of breaks in the film.

This process might cause a total loss of P.D. at either protoplasmic surface. If it occurred at one surface while the other had a negative P.D. the resultant P.D.⁸ would be negative.

(2) Production of organic ions in the protoplasm which lessen the outwardly directed potential.

(3) Changes in potentials not due to diffusion, e.g., in phase boundary potential or in membrane potential.⁹

Glucose, sucrose and maltose act like glycerol, as shown in table 1. One striking effect is common to all of these, namely, the recovery of the original P.D. on standing.¹⁰ For example, when sea water is replaced by sea water plus an equal volume of isotonic glycerol solution (containing $CaCl_2$ and KCl) the P.D. becomes less positive in the course of about 20 seconds to the extent of about 29 mv. But a few seconds later the P.D. begins to grow more positive again¹¹ as though the non-electrolyte were penetrating to the

inner protoplasmic surface and there setting up changes opposite in sign to those produced at the outer surface. Such changes at the two surfaces might very well be opposite in sign since the surfaces are known to differ greatly.¹² They would be possible even if the surfaces were alike provided ions produced in the protoplasm diffused inward as well as outward.

TABLE 1

CHANGE OF P.D. PRODUCED BY REPLACING SEA WATER IN CONTACT WITH *Halicystis* BY SEA WATER PLUS AN EQUAL VOLUME OF AN ISOTONIC SOLUTION CONTAINING 1.1 M NON-ELECTROLYTE + 0.02 M CaCl_2 + 0.012 M KCl.* ALL AT pH 8.2 UNLESS OTHERWISE STATED

NON-ELECTROLYTE IN SUBSTITUTED SOLUTION	CHANGE IN P.D.	NUMBER OF OBSERVATIONS†
Glycerol	28.9 ± 0.83	5
Glucose	27.8 ± 4.74	6
Sucrose	17.1 ± 1.56	6
Maltose	38.0 ± 1.59	13
Mannite	13.8 ± 0.52	14
Mannite pH 6.4	9.8 ± 0.68	13

* In each case the P.D. became less positive (the sign is positive when the positive current tends to flow outward from the sap to the external solution).

† A limited number of cells was available.

In the course of 5 minutes or less the P.D. usually returns approximately to the original value in sea water. If the cell is then transferred to sea water little or no change in P.D. occurs.¹³ This may mean that the glycerol is washed out of both surfaces at about the same rate so that the changes in P.D. at one surface are cancelled by those at the other.

A different picture is presented when we employ mannite.

(1) With mannite the theoretical limit is not as a rule exceeded. The average loss of P.D. when sea water is replaced by sea water plus an equal volume of 1.1 M mannite + 0.02 M CaCl_2 + 0.012 M KCl at pH 8.2 is about 14 mv. An occasional measurement runs higher¹⁴ (up to 22 mv.). Experiments at pH 6.4 gave a lower value (table 1). These were made by lowering the pH of the sea water from 8.2 to 6.4 and then transferring to the dilute sea water at 6.4. The low value may be connected with the fact that the P.D. is reduced by lowering the pH of the sea water (this will be discussed in a subsequent paper).

(2) The P.D. thus produced remains constant for 8 minutes or more. It does not as a rule increase again and show "recovery."¹⁵

In view of this it would seem advisable to use mannite in studying the concentration effect. We cannot be sure that it does not affect the values obtained but it appears to do so less than the other substances mentioned.

The high value obtained for the concentration effect with mannite is of interest. If we use this value to calculate $U_{\text{Na}} + V_{\text{Cl}}$ we obtain a value very much less than that found for *Valonia*¹ (in *Valonia* $U_{\text{Na}} \div V_{\text{Cl}} = 0.2$).

To account for the values in table 1 in excess of the theoretical, the simplest assumption seems to be that partition coefficients are altered by all the non-electrolytes except mannite. Unfortunately the theory¹⁶ of partition coefficients is not yet developed and at present we can do little more than record suggestive facts. If non-electrolytes can alter the partition coefficients of electrolytes it is evident that this may be of importance for the cell.

It would seem that if glycerol lessens the partition coefficients of electrolytes, as suggested, the addition of glycerol to sea water should make the P.D. less positive. This is the case. Enough glycerol was added to sea water to increase the osmotic pressure by about 50 per cent. Enough solid NaCl was then added to bring the halide content up to the normal (0.58 M). In the course of 3 to 5 minutes the P.D. became less positive by 10 to 20 mv. On replacing in sea water the P.D. returned to normal in 4 minutes or less, but in the meantime it became temporarily still less positive to the extent of 5 to 10 mv.

Enough mannite was added to sea water to raise its osmotic pressure about 50 per cent and solid NaCl was then added to make the halide content 0.58 M as usual. Since this produced no change in P.D. we might conclude that, as expected, mannite does not change the partition coefficient as glycerol does. But mannite is not wholly without effect on the P.D. for when the cells are replaced in sea water the P.D. becomes temporarily less positive to the extent of 10 to 15 mv. After this it returns to normal (the whole process takes about 4 minutes). The cause of these changes is presumably osmotic and is due to the taking up of water by the different parts of the protoplasm. As might be expected this is similar with glycerol and with mannite.

That the taking up of water can cause the P.D. to become less positive is suggested by experiments with sea water plus an equal volume of distilled water. In this the P.D. became within 15 seconds 44 ± 3 mv. less positive (10 observations).¹⁷ Similar experiments performed by L. R. Blinks yielded smaller values.¹⁸

Replaced in sea water the cells returned in 4 minutes or less to the normal P.D.¹⁹ In this case we appear to have an effect due to change of electrolyte concentration plus an effect due to the redistribution of water. The effect is therefore much greater than that produced by mannite.

Summary.—To study changes in P.D. caused by diluting the sea water bathing cells of *Halicystis* it is desirable to add a non-electrolyte to maintain the osmotic pressure of the external solution. For this purpose mannite appears to be one of the most suitable. With glycerol, glucose, sucrose and maltose the changes of P.D. are so large that a reversible alteration of the protoplasmic surface is indicated. This may affect the P.D. by changing the partition coefficients of electrolytes or in other ways.

¹ Damon, E. B., *Jour. Gen. Physiol.*, **13**, 445 (1929-30).

² Osterhout, W. J. V., *Ibid.*, **13**, 715 (1929-30).

³ The experiments were done with *Halicystis Osterhoutii* (Blinks, L. R., and Blinks, A. H., *Bull. Torrey Bot. Club*, **57**, 389 (1931)), using the technique described in a previous paper (Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20**, 13 (1936-37)). Temperature about 22°C.

Unless otherwise stated there was no appearance of injury during the treatment or in the following days.

⁴ Blinks, L. R., *Jour. Gen. Physiol.*, **13**, 223 (1929-30); **18**, 409 (1934-35).

⁵ There is a latent period of 15 seconds or less. This is very variable and may depend to some extent on the thickness and cutinization of the cellulose wall and on bacterial jelly covering the cell. Such jelly gives the cells a slippery feeling. There seemed to be no jelly on these cells.

This latent period was also observed in experiments with KCl, NH₄Cl at pH 8.2 (0.005 M but not with 0.3 M), and 0.01 M guaiacol.

⁶ See table 1. A similar result was previously obtained by L. R. Blinks (personal communication).

⁷ Cf. Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20**, 13 (1936-37).

⁸ For reversal of sign of *Halicystis ovalis* in unbalanced NaCl see Blinks, L. R., Rhodes, R. D., and McCallum, G. A., *Proc. Nat. Acad. Sci.*, **21**, 123 (1935). For reversal in *Valonia* caused by dilute sea water see Damon, E. B., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **13**, 457 (1929-30).

⁹ Cf. Teorell, T., *Proc. Soc. Exp. Biol. Med.*, **33**, 282 (1935), Meyer, K., and Sievers, J.-F., *Helv. Chim. Acta*, **19**, 987 (1936).

¹⁰ This has been observed with glycerol by L. R. Blinks (personal communication).

¹¹ This does not happen in *Valonia*.

¹² Cf. Blinks, L. R., *Jour. Gen. Physiol.*, **13**, 223 (1929-30); **18**, 409 (1934-35).

¹³ If the cell is transferred to sea water when the P.D. is at the minimum (i.e., before "recovery" has started) the P.D. becomes temporarily less positive to the extent of 4 mv. or less; this does not last more than a minute and the P.D. then returns to the normal value. This also applies to mannite. Apparently it does not apply to glucose. Presumably this means a different behavior at the inner and outer protoplasmic surfaces.

¹⁴ Blinks found that a lack of balance in the external solution made the P.D. less positive or even strongly negative. This may play a rôle here.

Cells injured by exposure to a temperature below 14°C. showed no change in P.D. when exposed to sea water plus an equal volume of mannite solution but when glycerol was used in place of mannite they showed the expected change. In both cases the cells were dead the next day. A few lots of cells showed no change in P.D. when transferred to sea water plus an equal volume of mannite solution even though they seemed normal in every other respect. One such lot when tested two days later gave the usual response.

¹⁵ An occasional cell shows a small decrease. Such cells become temporarily less positive when returned to sea water, as in the case of cells exposed to glycerol, but this temporary change is much less than with glycerol.

¹⁶ Cf. Shedlovsky, T., and Uhlig, H. H., *Jour. Gen. Physiol.*, **17**, 549, 563 (1933-34); Falkenhagen, H., *Electrolytes*, Oxford, Clarendon Press, 1934.

¹⁷ If left in the solution 30 seconds or more the P.D. began to increase (as with glycerol) but in most cases the cell was returned to sea water after 20 seconds.

¹⁸ Personal communication.

¹⁹ After the cell was replaced in sea water the P.D. did not become temporarily less positive as in the case of mannite and glycerol.

*A COMPARISON OF THE DIFFUSIBLE SUBSTANCES
CONCERNED WITH EYE COLOR DEVELOPMENT IN
DROSOPHILA, EPHESTIA AND HABROBRACON**

By G. W. BEADLE, R. L. ANDERSON AND JANE MAXWELL

STANFORD UNIVERSITY, JOHNSON C. SMITH UNIVERSITY, DANA HALL SCHOOL AND THE
MARINE BIOLOGICAL LABORATORY

Communicated December 22, 1937

Diffusible substances have been postulated as being concerned in eye color development in insects belonging to three orders, *Ephestia*¹ and *Bombyx*² (Lepidoptera), *Drosophila*³ (Diptera) and *Habrobracon*⁴ (Hymenoptera). It is known that the substances concerned in *Ephestia* and *Drosophila* are not species-specific.^{5,6} It is a matter of some interest to determine the relation to one another of the hormones or hormone-like substances in these insects. For example, is the "A-hormone" of *Ephestia* identical with either "*v*⁺ substance" or *cn*⁺ substance of *Drosophila*?⁷ In connection with this example, Ephrussi⁸ has given a comparative summary of the information concerning the A-hormone of *Ephestia* and the diffusible substances of *Drosophila*. In the present communication tests of *Ephestia* for substances active in *Drosophila* are reported; other experiments planned to show the relation between substances present in *Drosophila* and those in *Habrobracon* are described.

Material and Methods.—In the tests of *Ephestia*, wild type larvae, regularly used as hosts in culturing the parasitic wasp *Habrobracon*, were used. In making tests in *Drosophila*, vermilion brown (*v bw*) animals were used as a test for *v*⁺ substance and cinnabar brown (*cn bw*) animals as a test for *cn*⁺ substance. Wild type *Drosophila* pupae were used as a source of *v*⁺ and *cn*⁺ substances. Cinnabar pupae were similarly used as a source of *v*⁺ substance alone. In tests of *Habrobracon*, six eye color types were used: wild, orange (*o*), ivory (*o'*), red (*rd*), cantaloup (*c*) and white (*wh*). We are grateful to Doctor P. W. Whiting, who kindly placed these stocks of *Habrobracon*, as well as the material of *Ephestia*, at our disposal.

In all cases in which *Habrobracon* was used as a source of extracts (table 1) these extracts were made by heating larvae and pupae, taken shortly after cocoon formation, to 100°C. for a half minute or so, crushing and then removing the fluid fraction by centrifuging through a glass wool and asbestos filter.⁹ Such extracts were tested by injection into *Drosophila* test larvae in amounts of approximately 0.5 to 1.0 cubic mm. per larva. In certain tests, designated in table 1, a feeding technique was used.¹⁰ From 25 to 75 larvae and pupae (used shortly after spinning of cocoons) were heated to 100°C. for about 40 seconds, crushed and fed to 10 to 12 test larvae of *Drosophila* some 60 to 72 hours after egg laying (25°C.). The extract

of cinnabar *Drosophila* pupae was prepared in essentially the same way as described above for *Habrobracon*. The extracts of wild type pupae injected into ivory *Habrobracon* were prepared in a more elaborate way by Doctor Edward L. Tatum, to whom we are grateful for making them available.

TABLE 1
MODIFICATIONS OF THE EYE COLOR OF *DROSOPHILA* BY DIFFUSIBLE SUBSTANCES FROM
HABROBRACON AND THE RECIPROCAL

SOURCE OF SUBSTANCES	RECIPIENT	METHOD OF TREATMENT	NUMBER OF SEPARATE TESTS	NUMBER OF TEST ANIMALS POSITIVE	NUMBER OF TEST ANIMALS NEGATIVE	STRENGTH OF EFFECT
Wild Hab.	<i>v bw</i> Dros.	Extract injected	3	13	1	1.1
Same	Same	Fed	2	16	0	1.8
Wild Hab.	<i>cn bw</i> Dros.	Extract injected	3	14	1	1.0
Same	Same	Fed	3	23	0	2.0
Orange Hab.	<i>v bw</i> Dros.	Extract injected	2	9	0	1.6
Same	Same	Fed	2	17	0	1.9
Orange Hab.	<i>cn bw</i> Dros.	Extract injected	2	0	9	0.0
Same	Same	Fed	2	0	16	0.0
Ivory Hab.	<i>v bw</i> Dros.	Extract injected	1	14	0	2.3
Same	Same	Fed	2	18	0	2.5
Ivory Hab.	<i>cn bw</i> Dros.	Extract injected	1	0	16	0.0
Same	Same	Fed	2	0	18	0.0
Red Hab.	<i>v bw</i> Dros.	Extract injected	1	8	0	3.0
Same	Same	Fed	1	11	0	2.2
Red Hab.	<i>cn bw</i> Dros.	Extract injected	1	7*	0	0.1
Same	Same	Fed	1	10	0	2.1
White Hab.	<i>v bw</i> Dros.	Extract injected	1	5	0	1.0
Same	Same	Fed	2	16	0	2.0
White Hab.	<i>cn bw</i> Dros.	Extract injected	1	7*	0	0.2
Same	Same	Fed	2	9*	13*	0.2
Cantaloup Hab.	<i>v bw</i> Dros.	Fed	1	8	0	3.1
Cantaloup Hab.	<i>cn bw</i> Dros.	Fed	1	9	0	2.8
Wild Dros.	Ivory Hab.	Purified extract injected	2	32	2	brown
Cinnabar Dros.	Ivory Hab.	Extract injected	1	0	7	none
Cinnabar Dros.	<i>v bw</i> Dros.	Extract injected	1	12	0	1.0
(Control for above experiment, same extract used)						
Ringer's solution	Ivory Hab.	Injected	1	0	6	none
(operation control)						

* Classification as "positive" or "negative" not certain.

Strengths of tests are recorded according to an arbitrary numerical scale, 0 representing no modification and 5 the maximum change possible. It

should be emphasized that the numerical values obtained in different tests are not strictly comparable; to be so, tests must be run in parallel and the test animals directly cross-compared.

Tests of Ephestia.—One apparent difference between the A-hormone of *Ephestia* and either v^+ or cn^+ substance of *Drosophila* is the fact that A-hormone is produced and released by gonad transplants¹ whereas neither v^+ nor cn^+ substance appears to be released by such transplants in *Drosophila*.¹¹ Extracts were made of larval testes of *Ephestia* by heating the excised testes in Ringer's solution. Such extracts gave a positive test for both v^+ substance (21 test animals, all positive, average color value 1.5) and cn^+ substance (13 test animals, all positive, average color value 1.7). Similar tests of larval testes of *Drosophila* are negative for both substances (15 and 10 individuals in tests for v^+ and cn^+ substances). Transplanted testes of wild type *Drosophila* are likewise without effect on the eye color of either vermilion brown or cinnabar brown hosts (11 and 10 individuals with well developed implants in the respective two tests). It is thus clear that extracts of *Ephestia* testes contain substances similar in effects to those found in *Drosophila*. These facts, in connection with the general similarity in effects of A-hormone and the *Drosophila* substances and the similarities in solubility properties,^{9,12,13} suggest that the A-hormone might well be identical with either v^+ or cn^+ substance. Appropriate tests of the red eyed mutant of *Ephestia*, not available to the authors, should show with which, if either, of the *Drosophila* substances the A-hormone might be identical.

Tests Involving Habrobracon.—It has been shown that in certain types of double nucleus mosaics in *Habrobracon* the eye color characters orange and ivory (differentiated by the genes o and o^i , members of an allelic series) are non-autonomous.³ There appears to be a diffusion of some substance from orange-plus (o^+) to orange (o) or ivory (o^i) tissue such that orange or ivory eye tissue is modified in the direction of wild type (black). If this assumed substance is identical with one or the other of the eye color substances known in *Drosophila*, it should be possible to demonstrate the presence of the substance in wild type *Habrobracon* by tests made on *Drosophila*. It should likewise be possible to demonstrate that the substance concerned is either absent or reduced in amount in orange or ivory *Habrobracon*.

Such tests have been made following the methods outlined in a previous section of this paper. The results are summarized in table 1. Extracts of wild type wasps are positive for both substances. Both orange and ivory wasps gave extracts positive for v^+ substance but negative for cn^+ substance. These results suggest that the substance concerned in the differentiation of orange and ivory from wild type is similar to, or identical with, cn^+ substance of *Drosophila*. Reciprocal tests, extracts of *Drosophila*

pupae injected into *Habrobracon* larvae or pupae, show that wild type *Drosophila* pupae contain a substance capable of modifying ivory eye color toward wild type. Furthermore, an extract of cinnabar pupae, known from previous experience⁹ to contain v^+ substance but not cn^+ substance, was without apparent effect on the eye color of ivory *Habrobracon* pupae. A parallel test of the same extract of cinnabar pupae was made in vermilion brown larvae to determine whether v^+ substance was actually present in the particular extract used in *Habrobracon*; the test was positive.

Although the proof cannot be considered to be complete, the above facts do provide a reasonable basis for postulating that the substance deficient in orange or ivory wasps is the same as that deficient in cinnabar flies.

Three additional eye color types of *Habrobracon* have been tested for both v^+ and cn^+ substances. Red and cantaloup give tests for both substances as strong or stronger than wild type (table 1). Assuming that the indicated differences in the amount of substances are significant, it is suggested that the relation between pigment formation and utilization of diffusible substances found by Ephrussi and Chevais¹⁴ in *Drosophila* might account for them. Extracts of white *Habrobracon* larvae and pupae give positive tests for v^+ substances but are very low in activity in tests for cn^+ substance. In fact, cn^+ substance tests of white wasps are so weak that it is only by the most careful comparison with control test animals that a modification can be detected. On the basis of these tests alone, one would hesitate to differentiate between white and ivory. However, it is known from studies of mosaics that the white character is autonomous in development and in addition that white tissue in such mosaics is capable of producing and releasing a substance that modifies orange eye tissue in the same apparent manner as does cn^+ substance.⁴

Discussion.—The host-parasite relations between *Ephestia* and *Habrobracon* are of interest in relation to the eye color substances. *Ephestia* larvae contain both v^+ and cn^+ substances. It is known that both of these substances can be effectively administered to *Drosophila* by feeding.¹⁰ Furthermore, it has been shown that cn^+ substance (from *Drosophila*) will produce a modification in genetically ivory wasps when injected into larvae. Why does an orange or ivory *Habrobracon* larva, feeding on an *Ephestia* host, not obtain sufficient cn^+ substance to modify its eye color; in other words, how, under these conditions, can there be an orange or ivory character in *Habrobracon*? Several explanations are possible; for example, it is possible that cn^+ substance is not present in the blood of paralyzed *Ephestia* larvae or that it is not effective when taken in with food material as it is in *Drosophila*. Another possibility, and one that we consider more probable, is as follows: It has been postulated that both v^+ and cn^+ substances are inactivated in air by enzymic oxidation.⁹ If this is correct, the enzyme system concerned is present in *Drosophila* larvae and pupae.

It is probable, then, that this system is likewise present in *Ephestia* larvae. The parasitic *Habrobracon* larvae, according to this view, removes both cn^+ substance and the enzyme system from the host and it is at least possible that under these conditions the cn^+ substance obtained is inactivated in the intestine of the wasp larva.

Among more than twenty-five eye color characters studied in *Drosophila melanogaster*, differentiated from wild type by as many non-allelic genes, only one is both non-autonomous and differentiated from wild type by a marked deficiency (or absence) of cn^+ substance.¹⁵ Among the known eye color characters in *Habrobracon*, only the orange series is similarly non-autonomous and characterized by a deficiency or absence of a diffusible substance that gives the same reaction in *Drosophila* as does cn^+ substance. We are therefore tempted to suggest that the cn^+ and o^+ genes are homologous and that their mutant alleles represent parallel mutations. Similar arguments, though having a less substantial experimental basis, would suggest that the a^+ (*A*) gene in *Ephestia* is homologous with either the v^+ or the cn^+ gene in *Drosophila*. A more nearly adequate knowledge than we now have of the chemical processes involved in eye color development in these insects should provide a more satisfactory basis for these and similar inferences.

NOTE: After the manuscript of the present paper was submitted for publication, a paper appeared (E. Becker and E. Plagge, *Naturwiss.*, 25, 809 (1937)) in which it is shown that the red eyed mutant of *Ephestia* (*a*) is deficient in cn^+ substance (tested in *Drosophila*) and that wild type and cinnabar *Drosophila* contain a substance that modifies the red eyed mutant of *Ephestia* toward wild type. Although the direct test of the *a* mutant of *Ephestia* for v^+ substance remains to be made, it appears probable from the evidence now available that the a^+ (*A*) gene in *Ephestia* and the v^+ gene in *Drosophila* are homologous and that the recessive alleles (*a* and *v*) of these genes represent parallel mutations.

* That part of this work carried out at Stanford University was aided by funds granted by the Rockefeller Foundation.

¹ E. Caspari, *Arch. Entw.-mech.*, 130, 353-381 (1933); A. Kühn, E. Caspari and E. Plagge, *Nachr. Ges. Wiss. Göttingen*, 2, 1-29 (1935).

² H. Kikkawa, *Zool. Mag. (Japan)*, 49, 348-353 (1937).

³ A. H. Sturtevant, *Proc. Sixth Int. Cong. Genet.*, 1, 304-307 (1932); B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, 71, 54-74 (1937); G. W. Beadle and B. Ephrussi, *Genetics*, 22, 76-86 (1937).

⁴ P. W. Whiting, *Biol. Bull.*, 63, 296-309 (1932); P. W. Whiting and A. R. Whiting, *Jour. Genet.*, 29, 311-316 (1934).

⁵ B. Ephrussi and M. H. Harnly, *C. R. Acad. Sci., Paris*, 203, 1028 (1936).

⁶ E. Plagge, *Nachr. Ges. Wiss. Göttingen*, 2, 251-256 (1936).

⁷ Experiments as yet unpublished suggest that the so-called " ca^+ substance" of *Drosophila*¹⁶ does not exist as a distinct substance but that what may be called the "claret effect" is one aspect of the action of either v^+ or cn^+ substance.

⁸ B. Ephrussi, *Amer. Nat.* (in press).

⁹ K. V. Thimann and G. W. Beadle, these PROCEEDINGS, 23, 143-146 (1937).

¹⁰ G. W. Beadle and L. W. Law, *Proc. Soc. Exptl. Biol. Med.* (in press).

¹¹ B. Ephrussi and G. W. Beadle, *Bull. Biol. Fr. Belg.*, **69**, 492-502 (1935). Also unpublished results.

¹² Y. Khouvine, B. Ephrussi, and M. H. Harnly, *C. R. Acad. Sci. Paris*, **203**, 1542 (1936).

¹³ E. Becker, *Naturwiss.*, **25**, 507 (1937).

¹⁴ B. Ephrussi and S. Chevais, these PROCEEDINGS, **23**, 428-434 (1937).

¹⁵ G. W. Beadle and B. Ephrussi, *Genetics*, **21**, 225-247 (1936).

THE EFFECT OF pH ON THE DEVELOPMENT OF ULTRA-CENTRIFUGED FUCUS EGGS¹

BY D. M. WHITAKER

SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated December 19, 1937

It has been shown (Whitaker²) that the developmental polarity of fertilized eggs of *Fucus furcatus* is determined by centrifuging at 3000 x g. for 15 or 20 minutes. When the stratified eggs are reared in the dark in normal sea water (pH 7.9-8.0), more than 99% form rhizoids on the centrifugal halves of the eggs, and 93-99% do so within 10° of the centrifugal pole.

Beams³ ultra-centrifuged fertilized eggs of *Fucus serratus* at 150,000 x g. for half an hour and found the polarity to be unaffected by stratification of the visible cell inclusions. It is possible but not probable that the difference lies in the two species of *Fucus*. It also appeared possible that the effects of centrifuging at 3000 x g. might be lost at 150,000 x g. Might some substance or structure be moved at 3000 x g. but be broken down at 150,000 x g.? Thousands of *F. furcatus* eggs were ultra-centrifuged in quartz centrifuge tubes at various forces including 150,000 x g. and 200,000 x g. for various durations, including 5 minutes and half an hour. The eggs were grown in normal sea water (pH 7.8-8.1) in the dark in a constant temperature room at 15°C. in thin cultures to avoid group effect (Whitaker⁴). Invariably 99% formed rhizoids on the centrifugal halves of the eggs, about 90% or more doing so within 10° of the centrifugal pole. The results are therefore essentially the same at 150,000 x g. and at 3000 x g., except that at the higher force 5 minutes of centrifuging is more than adequate to sharply stratify the eggs and determine polarity.

A number of environmental factors affect the determination of polarity in the *Fucus* egg.^{3,4} When ultra-centrifuged eggs of *F. furcatus* are illuminated from one side during development, the polarity is affected by the direction of the light as well as by the stratification⁵ so that in a population rhizoids are observed in all positions with respect to the stratification. The polarity of ultra-centrifuged eggs is also altered by the group effect, if

neighboring eggs are close together, especially if the sea water is somewhat acid. It will be shown below that the response of an isolated egg to its internal stratification depends on the pH of the medium. Beams does not indicate the conditions of his experiments with respect to these factors, and one or a combination of these factors would be adequate to explain why he observed rhizoids in all positions with respect to stratification.

The principal purpose of this communication is to present the results of experiments designed to test the effect of the pH of the external medium on the polarity of centrifuged *Fucus* eggs. A more detailed report will be published elsewhere. Eggs were fertilized and centrifuged in filtered normal sea water. They were centrifuged for 5 minutes at 150,000 x g., beginning 10–20 minutes after fertilization, and were then grown individually in 1 cc. sea water in small individual syracuse dishes in a dark constant temperature room at 15°C. Three hundred and ninety-six eggs were grown in filtered normal sea water, initially at pH 7.9–8.1, and 339 eggs were grown in sea water acidified to pH 5.8–6.1 with McIlvaine's buffer. One hundred per cent of the eggs reared at pH 7.9–8.1 formed rhizoids on the centrifugal halves. Only 10% of the eggs reared at pH 5.8–6.1 formed rhizoids on the centrifugal halves and 90% formed rhizoids instead on the centripetal halves. A considerable number formed rhizoids at the centripetal pole. The developmental response of the egg to its internal stratification is thus reversed with change in external pH. Centrifuged eggs reared at pH 6.0 in mass cultures which were thin so that mutual influences were reduced, but not absent, also statistically showed the reversal, but to a lesser extent.

Only tentative interpretations can be suggested at the present time. It was pointed out earlier^{4,6} that the responses of the *Fucus* egg to a variety of agents could be interpreted on the basis of plant growth hormone (auxin) as one of the links in the reaction chain leading to rhizoid formation. The auxin interpretation is strengthened by the recent work of du Buy and Olson⁷ who report having extracted growth substance from *Fucus* eggs. Olson and du Buy⁸ also carried out experiments from which they conclude that the rhizoid forms on the side of the egg to which beta-indole acetic acid (hetero-auxin) or its potassium salt is applied in sufficiently high concentration. Auxins are active in the molecular form, and hydrogen ions convert dissociated auxin into molecular auxin. If auxin in the egg is concentrated at the centrifugal end as a result of being adsorbed or attached to larger particles which are moved by centrifuging, the behavior of centrifuged eggs at pH 8.0 can be understood. The reversed response at pH 6.0 could perhaps be explained on this basis by supposing that after acid activation the active auxin at the centrifugal pole is now present in such high concentration as to be inhibitory so that the rhizoid forms at a region of lesser auxin concentration, i.e., more centripetally. Another possibility is that

an amphoteric phenomenon is responsible for the reversal, either through its effect on auxin or through more direct effect on the underlying rhizoid forming processes. Auxin may be unmoved by the centrifuge and amphoteric substances (e.g., protein) concentrated at the centrifugal end may affect either the activity or transport of auxin in a manner which would reverse on either side of the isoelectric point of the amphoteric substances. Both internal pH and electrical gradients might be expected to reverse when the isoelectric point is crossed if amphoteric substances are concentrated at one end of the cell. Experiments are in progress which it is hoped may throw further light on these questions.

¹ This work has been supported in part by funds granted by the Rockefeller Foundation. The author is indebted to Dr. E. W. Lowrance for assistance in carrying out the experiments.

² Whitaker, D. M., *Biol. Bull.*, **73**, 249 (1937).

³ Beams, H. W., *Jour. Marine Biol. Assn.*, **21**, 571 (1937).

⁴ Whitaker, D. M., *Jour. Gen. Physiol.*, **20**, 491 (1937).

⁵ As shown earlier in *Cystosira* with low centrifugal force by Knapp, E., *Planta*, **14**, 731 (1931).

⁶ Whitaker, D. M., and E. W. Lowrance, *Jour. Gen. Physiol.*, **21**, 57 (1937).

⁷ du Buy, H. G., and R. A. Olson, *Am. Jour. Bot.*, **24**, 609 (1937).

⁸ Olson, R. A., and H. G. du Buy, *Ibid.*, **24**, 611 (1937).

CHROMOSOME NUMBERS IN NODULES AND ROOTS OF RED CLOVER, COMMON VETCH AND GARDEN PEA¹

BY LOUISE WIPF AND D. C. COOPER

DEPARTMENTS OF AGRICULTURAL BACTERIOLOGY AND GENETICS, UNIVERSITY OF WISCONSIN

Communicated December 27, 1937

An observation of 28 chromosomes ($4n$) on somatic equatorial plates in the meristematic region of the nodule of red clover instead of the 14 chromosomes ($2n$) found in root tip cells suggested the present investigation. The number of chromosomes characteristic of nodular tissue has heretofore been reported in two instances. Milovidov² found 42 chromosomes in cells of the cortex of the nodule of *Lupinus mutabilis*. Lechtova-Trnka³ observed approximately 16 chromosomes in nodular material of both *Sophora Moorcroftiana* and *Robinia viscosa*. The exact number was difficult to determine because of the large size and crowded condition of the chromosomes on the equatorial plate.

Nodules and root tips of red clover (*Trifolium pratense* L.), common vetch (*Vicia angustifolia* L.) and garden pea (*Pisum sativum* L.) were used in the present study. They were obtained from field-grown plants and from



EXPLANATION OF PLATE

Figures 1-3. Somatic chromosomes from root tip cells. Fig. 1—Red clover ($2n = 14$). Fig. 2—Common vetch ($2n = 12$). Fig. 3—Garden pea ($2n = 14$).

Figures 4-6. Chromosome complements from nodule tissue. Fig. 4—Red clover (28 chromosomes). Fig. 5—Common vetch (24 chromosomes). Fig. 6—Garden pea (28 chromosomes). $\times 2150$.

plants grown in the greenhouses at the University of Wisconsin. Of the latter plants, some were grown in sterilized soil with the addition of controlled nutrients. The seeds were sterilized before planting and the young plants inoculated with an effective strain of the proper *Rhizobium* at the time of germination. The remaining plants were grown in an ordinary greenhouse soil mixture and, as with the field-grown material, the nodules resulted from chance inoculation.

Root tips and nodules of varying ages were fixed in Karpechenko's modification of Navashin's fluid and in Carnoy's alcohol-acetic-chloroform solution. The material fixed in Karpechenko's fluid was embedded in paraffin and sectioned. Aceto-carmin smears were made from that fixed in Carnoy's solution. The latter method was found to be more convenient for the determination of the chromosome numbers in the nodules. The mitotic spindles occur in irregular positions in the meristematic region, and the smear method makes it possible to obtain a higher percentage of polar views of equatorial plates than the sectioned material reveals. Transverse sections of root tips gave an abundance of figures wherein an exact count of the chromosomes was possible. Nodules from five plants of each species were examined (table 1).

TABLE 1
SHOWING NUMBER OF CHROMOSOMES IN THE NODULES OF RED CLOVER, COMMON VETCH
AND GARDEN PEA

PLANT	NODULE	RED CLOVER — $2n = 14$		COMMON VETCH — $2n = 12$		GARDEN PEA — $2n = 14$	
		NUMBER OF COUNTS	NUMBER OF CHROMOSOMES	NUMBER OF COUNTS	NUMBER OF CHROMOSOMES	NUMBER OF COUNTS	NUMBER OF CHROMOSOMES
1	a	1	28	1	24	2	28
	b	9	28	4	24	2	28
	c	1	28	1	24	2	28
2	a	5	28	4	24	2	28
3	a	2	28	2	24	4	28
4	a	3	28	2	24	31	28
	b ¹	5	28	1	24	42 ²	14
5	a	1	28	7	24	2	28
	b	1	28	14	24	6	28
				1	12 ³		
Totals	9	28	28	36	24	51	28

¹ Of the garden pea only 8 nodules were used.

² One count showed 12 chromosomes.

³ 73 chromosome counts were made from one nodule; 42 showed 14 chromosomes, 31 showed 28 chromosomes. See text for explanation.

The somatic number of chromosomes of red clover as observed in the dividing cells of the root tip is 14 (figure 1), whereas that of the cells in the nodule is 28 (figure 4). Counts were made in aceto-carmin preparations from 9 nodules (table 1). Polar views of 28 equatorial plates in nodular

tissue revealed regularly the tetraploid condition. Anaphase figures showed 28 chromosomes passing to each pole. In no case as yet has other than the tetraploid number appeared.

The diploid chromosome number in the vetch is 12 (figure 2). Twenty-four chromosomes are usually present on equatorial plates in the dividing cells of the nodule (figure 5). A total of 36 counts taken from 9 nodules revealed this number (table 1). Only one figure was observed with the diploid number (12).

Fourteen chromosomes are present in root tip cells of the pea (figure 3). The tetraploid number (28) was observed on 51 equatorial plates in 8 nodules (figure 6). One nodule having a large number of dividing cells showed both diploid and tetraploid complements (table 1). The tetraploid figures were in cells whose cytoplasm stained deeply whereas the cytoplasm surrounding the diploid equatorial plates took up little of the stain. Transverse sections of the apical meristematic portion of a pea nodule reveals a definite arrangement of the two types of cells. The central portion of the nodule is composed of tetraploid cells. This is surrounded by several layers of cortical cells which are diploid. Median sections of the nodule show a central cylinder of large cells packed with bacteria. There is no evidence of the presence of bacteria in the smaller cells of the cortical region. The nuclei of the invaded cells, also, are distinctly larger than those of the cortex. Nuclear and cell size are commonly, as shown by Gates⁴ and others, increased with an increase in the chromosome number.

The cells of stimulated growths and hypertrophies of various types have, in some instances, been found to possess chromosome numbers differing from that typical of the species. Tetraploid tomato and *Solanum* plants have been produced from budding callus tissue, a fact taken to indicate that the cells of such tissue may oftentimes be tetraploid rather than diploid (Jørgensen;⁵ Lindstrom and Koos⁶). Diploid, tetraploid and octoploid cells have been observed in crown gall tissue of the sugar beet (Winge⁷) and of beet and tobacco (Levine⁸). Kostoff and Kendall⁹ find polyploid cells in crown gall tissue, in spontaneous tumors on hybrid tobacco plants and in tumors caused by chemical substances. Whitaker¹⁰ reports an occasional tetraploid condition in nonparasitic tumors on grafted tobacco. A varying number of chromosomes ranging from haploid to tetraploid or even higher is present in the cells of carcinomatous tissue in the fowl, rat and man (Levine⁸). In comparison with these findings, observations thus far indicate that in nodules of red clover, common vetch and garden pea the infected cells are regularly tetraploid.

¹ Herman Frasch Foundation in Agricultural Chemistry, Frasch Paper No. 148. Joint contribution from the Departments of Agricultural Chemistry and Agricultural Bacteriology and from the Department of Genetics, Agricultural Experiment Station,

No. 227, and the Department of Botany, University of Wisconsin. Published with the approval of the Director of the Station.

- ² Milovidov, P. F., *Centbl. Bact.* (etc.) 2 Abt., **68**, 333-345 (1926).
- ³ Lechtova-Trnka, M., *Le Botaniste*, **23**, 301-530 (1931).
- ⁴ Gates, R. R., *Arch. Zellforsch.*, **3**, 525-552 (1909).
- ⁵ Jørgensen, C. A., *Jour. Genetics*, **19**, 133-211 (1928).
- ⁶ Lindstrom, E. W., and Koos, Katherine, *Amer. Jour. Bot.*, **18**, 398-410 (1931).
- ⁷ Winge, O., *Ztschr. Zellforsch. mikros. Anat.*, **6**, 397-423 (1927).
- ⁸ Levine, Michael, *Jour. Cancer Research*, **14**, 400-425 (1930).
- ⁹ Kostoff, D., and Kendall, J., *Arch. Mikrobiol.*, **4**, 487-508 (1933).
- ¹⁰ Whitaker, T. W., *Jour. Arnold Arboretum*, **15**, 144-153 (1934).

GROUPS HAVING A MAXIMUM SET OF INDEPENDENT GENERATORS OF THE SAME ORDER

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated December 16, 1937

Suppose that a given group G has a maximum number set of independent generators, that is, a set which involves as many operators as there are prime factors in the order of G . Obviously each of the operators of such a set is of prime order.¹ It is known that these operators can always be so selected that their product is equal to the order of G , and when they have been so selected and the order of G is not a power of a prime number then they can be separated into distinct subsets such that each subset generates a Sylow subgroup of G and that each of these Sylow subgroups is abelian and of type 1^k . When G is non-abelian only the Sylow subgroup whose order is a power of the largest prime number which divides the order of G is always invariant under G , and when all the operators in a maximum number set of independent generators of G are of the same order this order is the smallest prime number which divides the order of G .

Suppose that G contains a maximum number set of independent generators of the same order. We proceed to prove that all the operators of larger prime orders contained in G are relatively commutative and hence they generate an invariant abelian subgroup of G . To prove this fact let s_1, s_2, \dots, s_l be a subset of the given maximum number set of independent generators of G such that this subset generates a Sylow subgroup of G . Every other operator in the given maximum number set when adjoined to this subset gives a set of generators of a subgroup of G whose order is divisible by a larger prime number p than the prime divisors of the order of the given Sylow subgroup. Hence this subgroup contains an operator t_1 of order p . Similarly we find another operator t_2 of prime order $q \geq p$. To

prove that t_1 and t_2 are commutative we may assume that $s_1 s_\gamma = t_1$ and that $s_2 s_\delta = t_2$ and consider the group generated by the four operators $s_1, s_2, s_\gamma, s_\delta$ contained in the given maximum number set of independent generators of G .

The order of this group is the product of four prime numbers and the group involves the two operators t_1 and t_2 . Two of these prime numbers are p and q and two of them divide the order of the given Sylow subgroup. Hence t_2 is transformed into a power of itself by each of the three operators s_1, s_2, s_γ . If this is not the first power its index belongs to an exponent which is equal to the common order of these operators. The product $s_1 s_\gamma$ cannot transform t_2 into a power whose index belongs to the same prime number since the order of $s_1 s_\gamma$ is a larger prime number. Hence it results that t_1 and t_2 are commutative. That is, *when all the operators of a maximum number set of independent generators of a group have the same order then all the operators of the group whose prime orders exceed this common order are relatively commutative and generate an invariant abelian subgroup of the group.*

From the preceding paragraphs it results that comparatively few of the groups which have a maximum number set of independent generators can also have such a set in which all the operators have the same order. Every abelian group whose independent generators are composed of operators of odd prime orders can be extended in at least one way so as to obtain a group which has a maximum number set of independent generators of the same order. One such extending operator is of order 2 and transforms every operator of this abelian group into its inverse. When there exists a prime number such that each prime factor of the order of this abelian group is congruent to unity with respect to this prime number then the extending operator may clearly have an order which is equal to this prime number. Such extensions can obviously be effected in various ways whenever the order of this abelian subgroup is divisible by more than one prime number.

Suppose now that G has a maximum number set of independent generators composed of operators of the same order p and that it contains a Sylow subgroup of this order which is generated by s . A maximum number set of independent generators of G which is such that the product of the orders of these generators is equal to the order of G contains then only one operator of order p while every one of its other operators is of higher order. Each of these other operators is the product of s and some operator of order p contained in the given maximum number set of independent generators. Since each of the operators of a new maximum number set of independent generators found in this way is transformed into powers of itself by s and all these operators besides s are relatively commutative there results the following theorem: *If a group has a maximum number set of independent generators composed of operators of the same order p and contains a Sylow subgroup of order p then it involves an invariant abelian subgroup whose inde-*

pendent generators are of prime orders such that each order is congruent to unity modulo p and all of the remaining operators are conjugate under the group.

While the smallest Sylow subgroup of a group which has a maximum number set of independent generators whose common order is p is obviously of order p there is no largest such Sylow subgroup when the subgroup generated by its operators of larger prime orders is given. This results directly from the fact that if G is a given group which has a maximum number set of independent generators of order p then the direct product of G and any abelian group of type 1^k whose order is a power of p is also such a group. Such direct products will be excluded in what follows. When this is done and the invariant abelian subgroup which is generated by the operators of prime orders larger than p in G is fixed it can be proved that the order of the Sylow subgroup of G whose order is a power of p cannot exceed p^m , where m is the number of the independent generators of prime orders of the given abelian invariant subgroup of G .

To prove this theorem it may be noted that if s_1, s_2, \dots, s_l ($l > m$) is a set of independent generators of a Sylow subgroup whose order is a power of p contained in G an additional independent generator of G is the product of one of these l operators and some other operator of order p contained in the given maximum number set of independent generators of G . These two operators generate a non-abelian subgroup of G whose order is the product of two prime numbers. We form such subgroups successively by using each time a different independent generator of the given Sylow subgroup of G , and continue this process until a set of m independent generators of prime orders greater than p of the given abelian invariant subgroup of G is found. The remaining $l - m$ independent generators of the Sylow subgroup of order p^l contained in G may be supposed to generate an invariant subgroup of G which has only the identity in common with the invariant subgroup generated by the set of $2m$ independent generators noted above.

The fact that the subgroup generated by these $l - m$ operators of the given maximum number set of independent generators of G is invariant under G can be proved by observing that it is the cross-cut of all the Sylow subgroups of G whose orders are powers of p . This may be proved by observing that when the given Sylow subgroup of order p^l contained in G is extended by an operator of order p not contained in this Sylow subgroup there results a group whose order is a prime number q times the order of this Sylow subgroup under which $l - 1$ of the independent generators of the given Sylow subgroup may be assumed to be invariant. This group contains q Sylow subgroups of order p^l which have a cross-cut of index q under all of these Sylow subgroups. When G contains more than $l + 1$ independent generators this process can be repeated until we arrive at the given invariant cross-cut of order p^{l-m} .

Since the two given invariant subgroups have only the identity in common and the product of their order is equal to the order of G it results that G is the direct product of these two subgroups. This proves the following theorem: *If a group has a maximum number set of independent generators composed of operators of the same order p and if its invariant abelian subgroup which is generated by its operators of prime orders larger than p has m such generators then the order of its Sylow subgroup whose order is a power of p cannot exceed p^m unless the group is a direct product such that one of its factor groups is abelian and involves only operators of order p besides the identity.* It may be noted that when the order of this Sylow subgroup is p^m then the group is the direct product of m non-abelian groups such that each of these groups is of order pq , q being a larger prime number than p . Moreover, every such direct product has a maximum number set of independent generators composed of operators of order p .

If the operators of a maximum number set of independent generators of a group G are so chosen that the product of their orders is equal to the order of G then it is known that the operators of the same order in this set generate a Sylow subgroup of G for each of the orders. These operators of the same order can usually be selected in various ways. In the case when they generate the Sylow subgroup whose order is a power of the smallest prime number which divides the order of G they may be replaced by any other possible set of independent generators of this Sylow subgroup but this is not generally the case as regards the subset of independent generators which generate another Sylow subgroup of G since in this case it is not necessary that the group generated by an operator of such a subset is transformed into itself by all the operators of a smaller order in the given maximum number set of independent generators of G .

¹ G. A. Miller, *Proc. Nat. Acad. Sci.*, **23**, 333-337 (1937).

GROUPS HAVING A MAXIMUM NUMBER SET OF CONJUGATE INDEPENDENT GENERATORS

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated January 10, 1938

If a group G has a maximum number set of independent generators composed of operators which are conjugate under G then all the operators of the set are of the same order and hence such a group is included among those which have a maximum number set of independent generators composed of operators of the same order.¹ In particular, all the operators of the set have for their order the smallest prime factor p of the order G , and G

contains an abelian invariant subgroup whose independent generators are composed of operators of prime orders greater than p such that each of these prime numbers is congruent to unity modulo p . If this subgroup is so selected that it includes all of the operators of G of prime orders greater than p then all of the remaining operators of G are of order p and the cyclic subgroups of order p generated by them constitute a complete set of conjugates under G . In particular, the order of G is not divisible by p^2 . Simple illustrative examples of such groups are the dihedral groups whose orders are the double of odd prime numbers.

From the preceding paragraph it results that if a group has a maximum number set of independent generators composed of operators which are conjugate under it then its Sylow subgroups which involve these operators are of prime order p . Moreover, if a group which has a maximum number set of independent generators composed of operators of the same order p has a Sylow subgroup of order p then it contains a maximum number set of independent generators composed of conjugate operators. On the other hand, if such a group contains a Sylow subgroup whose order is divisible by p^2 then it cannot contain a maximum number set of independent generators composed of conjugate operators since the order of its Sylow subgroups is the index under G of the subgroup generated by all its operators of prime orders greater than p . Hence there results the following theorem: *A necessary and sufficient condition that a group which has a maximum number set of independent generators of the same order has such a set in which all these operators are conjugate under the group is that it has a Sylow subgroup of this order.*

Two necessary and sufficient conditions that there is a group of a given composite order which has a maximum number set of conjugate independent generators are that this order is not divisible by the square of its smallest prime factor and that each of its other prime factors is congruent to unity with respect to this smallest prime factor. In particular, there is one and only one such group whose order is the double of an arbitrary odd number. This is the dihedral group whenever the order is not divisible by the square of an odd prime number and the generalized dihedral group whenever the order is divisible by such a square. When the order of G is odd and is not the product of two prime numbers there exists always more than one such group of this order but all of these possible groups are conformal. In one and only one of them each of the cycle subgroups of prime order greater than p is invariant under G .

When all the prime numbers which divide the order of G and exceed p are distinct and their number is n the number of these groups of a given order is equal to $(p - 1)^n - 1$. This results from the fact that an operator of order p to be adjoined to the given abelian subgroup which is generated by all the operators of G which are of prime order greater than p may be

assumed to transform one of these operators into an arbitrary power whose index belongs to an exponent which is equal to p . When some of the prime numbers greater than p which divide the order of G are equal to each other it is somewhat more difficult to determine the total number of these possible groups of a given order. In the special case when there are only two such equal prime factors of the order of G and this order is the product of three prime numbers it is not difficult to determine the number of these possible groups as follows: Let q be one of the two prime numbers which divide the order of G and exceed p while s is an operator of order p which is adjoined to the given invariant subgroup of order q^2 .

It may be assumed that s transforms one of the two independent generators of order q into an arbitrary power of itself such that the index of this power belongs to exponent p modulo q . The other independent generator of order q of the given invariant subgroup of order q^2 is transformed by s into one of the possible $p - 1$ powers of itself. The indexes of the $p - 1$ distinct powers of this independent generator of order q which belong to exponent p modulo q together with the identity constitute a group of order p modulo q . Hence the $p - 1$ powers into one of which s may transform the second of the two given independent generators of order q after the first one has been transformed have indexes which constitute a cyclic group of order $p - 1$. As this has one and only one operator of order 2 the given second independent generator can be transformed in $(p + 1)/2$ ways relatively to the first. That is, *there are exactly $(p + 1)/2$ groups of order pq^2 , p and q being prime numbers such that $q - 1$ is divisible by p , which separately have a maximum number set of conjugate independent generators.*

When the order of G is divisible by q^m , $m > 2$, but exceeds pq^n , the adjoined operator s transforms at least m of the cyclic subgroups of G separately into themselves and the subgroups of order pq^m are completely determined by the different powers into which s transforms the given m independent generators of order q . Such a group is obviously transformed into itself by operators which transform these m independent generators according to the symmetric group of degree m . A necessary and sufficient condition that s transforms each of the cyclic subgroups of order q in a subgroup of order q^k contained in G into itself is that s transforms each of the k operators in a set of independent generators of this subgroup into the same power of itself. If s transforms each of these k independent generators into the same power of itself but another independent generator of order q contained in G into a different power of itself then the group generated by these $k + 1$ independent generators of G contains one and only one invariant subgroup of order q besides those found in a given subgroup of order q^k .

It may be noted that while all of these groups of the same order are conformal there are also usually many of them which are not only con-

formal but have also the same number of conjugate cyclic subgroups of the same order. This is the case, for instance, as regards all except one of the $(p-1)^{n-1}$ groups of the same order noted above. We have therefore here systems of distinct groups which have a large number of properties in common besides being conformal. The number and the form of the operators of possible sets of independent generators are thus seen to throw considerable light on certain systems of groups of finite orders.

¹ Miller, G. A., *Proc. Nat. Acad. Sci.*, **23**, 333-337 (1937).

THE EXISTENCE OF A MINIMAL SURFACE OF LEAST AREA BOUNDED BY PRESCRIBED JORDAN ARCS AND PRESCRIBED SURFACES

BY R. COURANT

DEPARTMENT OF MATHEMATICS, NEW YORK UNIVERSITY

Communicated January 7, 1938

Plateau's problem with non-fixed boundaries can be solved by means of a general theorem concerning boundary values of harmonic functions. The following note proves this theorem and, as a consequence, the existence of a minimal surface of least area of the type of a circular disc bounded by a given Jordan arc and a closed portion of a continuous surface. The generalization to cases where the boundary may consist of any finite number of Jordan arcs and of point sets on different manifolds, and where the topological structure of the minimal surface is arbitrarily prescribed, will be given in a more detailed paper.¹

1. *Lemma.*—We consider a system of harmonic functions $x_i(u, v)$, $i = 1, \dots, n$, regular in the upper half plane $B: v > 0$, so that the "Dirichlet integral" for the potential vector \mathfrak{x} with the components x_i

$$D[\mathfrak{x}] = \frac{1}{2} \int \int_B (\mathfrak{x}_u^2 + \mathfrak{x}_v^2) du dv$$

is finite. Then there exists a quantity $l(h)$ and a quantity $\sigma(h)$ with

$$l(h) \rightarrow \infty \text{ and } \sigma(h) \rightarrow 0 \text{ for } h \rightarrow 0 \quad (1)$$

so that for each point u_0, v_0 in B at the distance $v_0 = h$ from the u -axis

$$|\mathfrak{x}(u, h) - \mathfrak{x}(u_0, h)| \leq \sigma(h) \quad (2)$$

holds for the whole segment S on $v = h$ defined by

$$|u - u_0| \leq h t(h). \quad (8)$$

Proof: If

$$D_h[\xi] = \epsilon(h)^4.$$

denotes the Dirichlet integral of ξ over the strip $0 < v < 2h$, we have $\epsilon(h) \rightarrow 0$ for $h \rightarrow 0$ because of the existence of $D[\xi]$. Let K_h be the circle of radius $\frac{1}{2}h$ around a point $u, v = h$ on the segment S ; then we have by the mean value theorem of potential theory

$$\xi_u(u, h) = \frac{4}{h^2\pi} \int \int_{K_h} \xi_u du dv$$

and hence by Schwarz's inequality

$$\xi_u(u, h)^2 \leq \frac{16}{h^4\pi^2} \cdot \frac{h^2\pi}{4} \int \int_{K_h} \xi_u^2 du dv \leq \frac{8}{h^2\pi} \epsilon^4(h) < \frac{9}{h^2} \epsilon^4(h).$$

This gives

$$|\xi(u, h) - \xi(u_0, h)| \leq \int_{u_0}^u |\xi_u(u, h)| du \leq |u - u_0| \frac{3}{h} \epsilon^2(h)$$

and therefore the Lemma follows with

$$t(h) = \frac{1}{\epsilon(h)}, \quad \sigma(h) = 3\epsilon(h).$$

2. *Theorem.*—Let $\xi_\nu(u, v)$ be a sequence of potential vectors regular for $v > 0$ with equally bounded Dirichlet integrals

$$D[\xi_\nu] \leq \frac{1}{2}L.$$

We suppose the boundary values of ξ_ν are on a closed manifold M , which means the distance of the point $\xi_\nu(u, h)$ to M , tends to zero with h —necessarily uniformly in u . We further suppose that for $\nu \rightarrow \infty$ the longest distance of points in M , to a closed manifold M tends to zero. We finally assume that ξ_ν converges to a potential vector ξ uniformly in each closed subdomain of the half plane B .³ Then the boundary values of ξ are on M .

Proof: With the denotation of the Lemma we have on the straight segment $S: v = h$ and $|u - u_0| \leq h t(h)$ the inequality (2). Because of the uniform convergence of ξ_ν to ξ on S we have for sufficiently large $\nu > \nu(h)$ on S :

$$|\xi(u, h) - \xi_\nu(u, h)| \leq \sigma(h),$$

hence on S :

$$|\xi(u_0, h) - \xi_\nu(u, h)| \leq 2\sigma(h)$$

and therefore for every δ with $0 < \delta < h$

$$|\xi(u_0, h) - \xi_v(u, \delta)| - 2\sigma(h) \leq |\xi_v(u, h) - \xi_v(u, \delta)|$$

and thus

$$|\xi(u_0, h) - \xi_v(u, \delta)| \leq 2\sigma(h) + \int_0^h \left| \frac{\partial \xi_v}{\partial v} \right| dv. \quad (4)$$

If $d_v(u, h)$ denotes the distance of the point $\xi_v(u, h)$ to M_v , and $d(u, h)$ the corresponding distance to M , we infer from (4) for sufficiently small δ , so that $d_v(u, \delta) < \sigma(h)$,

$$d_v(u_0, h) \leq 3\sigma(h) + \int_0^h \left| \frac{\partial \xi_v}{\partial v} \right| dv.$$

Integrating over S with respect to u and using Schwarz's inequality

$$\left(\int_R \int \left| \frac{\partial \xi_v}{\partial v} \right| dudv \right)^2 \leq h^2 l(h) \int \int \left(\frac{\partial \xi_v}{\partial v} \right)^2 dudv \leq h^2 l(h) L$$

for the rectangle

$$R : |u - u_0| \leq h l(h) \text{ and } 0 < v \leq h,$$

we have

$$hl(h)d_v(u_0, h) \leq 3hl(h)\sigma(h) + (h^2 l(h)L)^{\frac{1}{2}}$$

or

$$d_v(u_0, h) \leq 3\sigma(h) + \frac{1}{\sqrt{l(h)}} \sqrt{L}.$$

For $v \rightarrow \infty$ it follows immediately

$$d(u_0, h) \leq 3\sigma(h) + \frac{1}{\sqrt{l(h)}} \sqrt{L}$$

which proves our theorem on account of (1).

The following supplementary facts, not needed here but useful for other applications, may be noted:

COROLLARY 1. A corresponding theorem holds for domains B bounded by k circles.

COROLLARY 2. If ξ is not constant, in the passage to the limit additional boundary conditions in the form of inequalities are preserved, e.g., if a part of the u -axis by virtue of ξ_v corresponds monotonically to a Jordan arc γ , tending to a Jordan arc γ , then by ξ the same part of the u -axis corresponds monotonically to γ .

COROLLARY 3. If M_v and M extend to infinity but are closed in every closed spherical domain in the ξ -space, the theorem subsists except possibly for values u_0 which form a set of measure zero.

COROLLARY 4. The theorem subsists, if instead of the relations defining M_ν and M , we have relations between the coördinates x_i which in addition contain explicitly the coördinate u on the u -axis.

3. *The Plateau Problem.*⁴—In the n -dimensional x -space a closed manifold M may consist of a part of a surface T of $n - 1$ or less dimensions and of a Jordan arc γ joining two points A and B on T . We consider all surfaces $x(u, v)$ defined in the upper half plane $B : v > 0$, so that x is continuous and has piecewise continuous first derivatives in B and has the boundary values on M whereby to the segment $|u| \geq 1$ of $v = 0$ the arc γ may correspond in a continuous and monotonic way. We suppose that there exist admissible vectors with finite Dirichlet integral $D[x]$. Then the problem is to prove the existence of an admissible x for which the absolute minimum d is attained. From the paper quoted above it then follows directly that the solution represents a minimal surface.

We consider a minimizing sequence of potential vectors x_ν for which the boundary values of x_ν are on manifolds M_ν consisting of γ and a continuous arc γ'_ν , which tends to T with increasing ν . Because of the boundedness of $D[x_\nu]$ we can choose a subsequence x_ν converging, as in the theorem of Nr. 2, to a potential vector x with $D[x] \leq d$. By imposing a three point condition, or a similar condition, on x_ν , we can prevent x from being constant. As is seen, e.g., from the consideration of the previous papers, or from Corollary 2, x maps the segment $|u| \geq 1$ of the u -axis monotonically on γ , and the theorem of Nr. 2 shows that all the other boundary points of x are on T . Hence x is admissible, we have $D[x] = d$, and x solves our problem.

4. *Remarks.*—(a) If the surface T extends to infinity it may happen that the solution of the problem and even all the vectors of a minimizing sequence are not bounded but have a finite or even infinite number of spikes reaching to infinity. Our theory can be extended to such cases by means of Corollary 3 of Nr. 2.

(b). If the whole manifold M reduces to a Jordan curve, we come back again to the ordinary Plateau problem. Our theorem thus leads to a new version of the solution of this problem. However, it is an interesting fact that, in this case, one has to require expressly the monotonic character of the mapping of the u -axis to the simply described Jordan contour.

(c) The generality of the manifolds M considered in this note excludes a detailed analysis of the behavior of the minimal surfaces in the boundary points on T . The variational equation leads to a "natural boundary condition" expressing orthogonality in an average sense. But to prove, under suitable additional conditions for M , the existence and the strict orthogonality of a tangent plane in boundary points of S on M seems a problem not accessible to the methods of this note.⁵

¹ This problem corresponds to Douglas' generalization of Plateau's original problem, cf. the papers by Douglas, *Journ. Math. Phys.*, **15**, 55 ff (Feb., 1936) and 106 ff (June, 1936) and Courant, these PROCEEDINGS, June, 1936, 369 ff and *Ann. Math.*, **38**, 679 ff (1937). For the case of boundary planes, cf. also the doctoral thesis by E. Ritter, soon to appear.

² It is noteworthy that the Lemma and the following Theorem subsist if instead of harmonic functions general monotonic functions of u and v are admitted.

³ Incidentally, at least a subsequence of the ξ_r satisfying this last condition can always be found.

⁴ A knowledge of the first two sections of the paper in the *Ann. Math.* (see footnote 1) is required for the following.

⁵ Cf. the similar situation with the boundary value problem of elliptic partial linear differential equations. See Courant-Hilbert, *Meth. math. Physik*, Vol. II, Chapter 7.

THE CONTINUUMPROBLEM

BY FELIX BERNSTEIN

NEW YORK UNIVERSITY

Communicated January 4, 1938

1. The continuumhypothesis of Georg Cantor consists in the theoretical assumption that the continuum represents the second infinite potency following the potency of the enumerable set of the natural numbers in immediate succession.

In terms of the concept of Alephs, discovered by Cantor in an attempt to build up a system of infinite potencies in which immediate succession is secured, the hypothesis can be stated in the form of the equation

$$2^{\aleph_0} = \aleph_1.$$

It is well known that this problem has played the rôle of the central problem in the theory of aggregates as developed by Georg Cantor. D. Hilbert in 1900 has restated the problem and he gave later an exposition of his ideas concerning the problem in a paper constituting a different approach which has not yet led to a conclusive proof.

The object of the present paper is to offer a solution of the problem within a new system of postulates of the theory of aggregates. It will be shown that by adoption of two new postulates which we call the axiom of identity and the postulate of exclusion a proof of the continuumhypothesis can be given.

We accept as basis of the deductions, beside these postulates any of the established systems of the theory of aggregates (Zermelo, Adolf Fraenkel, v. Neumann and others).

2. We formulate the new postulates in the following way:

I. *The Axiom of Identity*.—Be M_1 a subset of the set M . If in our system no proof can exist that M_1 is a true subset of M , we assume identity of M_1 and M .

II. *Postulate of Exclusion*.—Be M_1 a subset of M . If the following conditions are fulfilled

a) M_1 is equivalent to M ,

b) M_1 is of the same ordertype as M ,

c) no element of the subset M_2 complementary to M_1 in M is given,

then we assume that no proof is possible by some fourth argument, that M_1 is a true subset of M .

If therefore the conditions a) b) c) are fulfilled we conclude with the aid of the axiom of identity that M_1 is identical with M .

We do not comment in this paper on the question of independence of these axioms from the system of Zermelo-Fraenkel, neither do we discuss here the compatibility of the new axioms with the old ones. We confine ourselves to the more immediate task to prove that these axioms will be sufficient to solve the continuumproblem, and reduce it to its axiomatic equivalent.

It shall, however, be noticed that the postulate of exclusion is sufficient to prove that the continuumhypothesis is true or independent. The axiom of identity excludes the latter possibility.

3. Be

$$\alpha_1, \alpha_2, \dots, \alpha_s, \dots \quad (1)$$

an enumerable sequence of numbers of the second class. The set T of all sequences (1) is equivalent to the continuum.

We introduce now a number of concepts and theorems which explain under which conditions the existence of sequences (1) different from the sequences of a given system t can or cannot be derived.

3.1. *Distinctionsets*. Be t a subset of the set T . Be (τ_s) a sequence different from each sequence of t . In each sequence of t there shall exist therefore at least one element ϵ_s different from the element with the same index s of the sequence (τ_s) .

We call the element ϵ_s a distinctionelement.

A set D of distinctionelements ϵ_s which contains at least one distinctionelement in each sequence of t , is called a sufficient distinctionset with regard to t .

D fulfills the following conditions:

(a) D contains at least one element in each sequence.

(b) the elements of D with the same index s form a subset D_s which does not have as element every number of the second class.

It follows immediately the THEOREM 1:

The existence of a distinctionset D fulfilling (a) and (b) is the internal condition with regard to the set t which has to be fulfilled, if a sequence (τ_r) different from every sequence of t shall exist. The condition is necessary and obviously sufficient.

3.2. *Overalldense Sets.*—We call the subset t of T overall dense in T if for every given number α of the second class a sequence of t exists with every element greater than α .

THEOREM 2. Every sufficient distinctionset D of an overall dense subset t contains numbers of the second class greater than any given number α of the class.

Since D contains elements of each sequence, the sequence in which each element is greater than α has an element in common with D which greater than α .

THEOREM 3. Under the same assumptions at least one index r exists so that D_r contains elements greater than any given α .

Proof: If indeed in the contrary no such r would exist, and α_r would be the upper limes of the numbers in D_r , the upper limes α of all α_r would be greater than any element of D contrary to Theorem 2.

THEOREM 4. Be M_{1r} the set of numbers contained in D_r and be M the set of all numbers of the second class. We have

$$a) M_{1r} \text{ equivalent } M$$

and

$$b) M_{1r} \text{ of same ordertype as } M.$$

Proof: a) Since D_r contains elements greater than any given α , it follows that M_{1r} is of power \aleph_1 . b) M_{1r} is a well ordered subset of the well ordered set M which is of minimum ordertype of his powerclass. Hence M_{1r} is also of minimum ordertype, and therefore of the ordertype of M .

3.3 Suppose that an overalldense subset t of T exists, for which no knowledge is given that a sequence (τ_r) exists which is different from all sequences of t . We state the

THEOREM 5. Under the assumptions made we have for at least one index

$$M_{1r} \equiv M$$

and

$$t \equiv T.$$

Proof: We assume that in the contrary a proof is possible that t is a true subset of T . Then a system of distinctionsets D should be defined fulfilling the conditions 3.1a) and b).

Because of 3.2 Theorem 4, for at least one index r , D_r exists so that the set M_{1r} fulfills the conditions a) and b) of the axiom of exclusion.

On the other hand no sequence (τ_s) different from all sequences of l is given. This means that no τ_s is given for any value of the index s , and in particular not for $s = \tau$.

Indeed the knowledge of some of the τ_s is meaningless, if it does not imply that they form a part of a sequence which is different from all sequences of l : the knowledge of such a sequence forms an undivisible unit. Hence we have in addition to *a*) and *b*):

c) a number τ_r different from all numbers of D_r , respectively. $M_{1,r}$ is not known. $M_{1,r}$ fulfills, therefore, the conditions of the axiom of exclusion and therefore is $M_{1,r} \equiv M$. In consequence no D_r can be found fulfilling 3.1*b*) so that D cannot be determined. No proof, therefore, is possible that the set l is a true subset of T and because of the axiom of identity we assume $l \equiv T$.

4. THEOREM 6. The power of the set of the overalldense sets l which are of power \aleph_1 is 2^{\aleph_1} .

Since the power of all subsets l of T of power \aleph_1 is $(2^{\aleph_0})^{\aleph_1} = 2^{\aleph_1}$ it is sufficient to prove that the set P of overalldense sets in which not more than one sequence occurs with the lower limit α , is already of power 2^{\aleph_1} .

Be $p\alpha$ the set of all sequences of lower limit α . The product P of the \aleph_1 sets $p\alpha$ is certainly not smaller than 2^{\aleph_1} and therefore of power 2^{\aleph_1} .

Existence of overalldense sets of power \aleph_1 for which no knowledge is given that they are true subsets of T :

Applying the axiom of choice on the product P^{\aleph_1} times, and adding the \aleph choice sets, we can select an overalldense set l of power \aleph_1 which is sufficiently arbitrary so that we have no knowledge that it is a true subset of T . Such set fulfills the conditions of Theorem 5.

This completes the proof of the equation $2^{\aleph_0} = \aleph_1$.

ON THE EXISTENCE OF A CLOSED CONVEX SURFACE REALIZING A GIVEN RIEMANNIAN METRIC

BY HANS LEWY

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF CALIFORNIA AT BERKELEY

Communicated January 14, 1938

Let σ_0 be the surface of the unit sphere and $(ds_0)^2$ the first fundamental form of Differential Geometry on σ_0 . Denote by $(ds_1)^2$ another given positive definite form defined at each point of σ_0 which in local parameters (u, v) reduces to

$$(ds_1)^2 = E(du)^2 + 2Fdu dv + G(dv)^2.$$

Suppose that the Gaussian curvature K_1 of the metric $(ds_1)^2$ is positive. Weyl¹ has attacked the following problem: Does there exist a closed convex surface σ_1 which may be mapped in a one-to-one way on σ_0 so that the first fundamental form of σ_1 is precisely $(ds_1)^2$? (Embedment problem in the large.)

Weyl shows first that, under certain conditions affecting the differentiability of E, F, G , one can indicate a family of metrics $(ds_\lambda)^2, 0 \leq \lambda \leq 1$,

$$(ds_\lambda)^2 = E_\lambda d(u)^2 + 2F_\lambda du dv + G_\lambda (dv)^2$$

in the parameters (u, v) on σ_0 , such that

- i) $E_\lambda, F_\lambda, G_\lambda$ depend analytically on λ ;
- ii) for all λ the Gaussian curvature K_λ of $(ds_\lambda)^2$ is positive everywhere on σ_0 ;
- iii) for $\lambda = 0$ and $\lambda = 1$, $(ds_\lambda)^2$ reduces to $(ds_0)^2$, respectively $(ds_1)^2$.

Weyl then proves: If there exists a surface σ_{λ_0} which solves the embedment problem of $(ds_{\lambda_0})^2$ for a certain value λ_0 of λ , then there also exists a sufficiently small neighborhood of λ_0 , say $|\lambda - \lambda_0| < \epsilon(\lambda_0)$, such that the embedment can be obtained for all λ of this neighborhood.

These facts alone don't permit to conclude the existence of a σ_λ for $\lambda = 1$, as the described continuity method does not exclude the possibility (π) that there may be a value λ^1 which cannot be reached from $\lambda = 0$ in finitely many steps. Weyl discusses this point in the second part of his paper, suggesting a line of attack and establishing certain estimates which he believes to be the clue to the solution. However these ideas have never been carried through.

I have been able by a different method² to eliminate (π) and thus to complete the solution of the embedment problem in the large. The following lines give the essential steps of the proof for the case of analytic E, F, G ; in this case $E_\lambda, F_\lambda, G_\lambda$ are analytic in u, v, λ .

A result of Darboux³ shows that the distance $\rho_\lambda(u, v)$ from a fixed point P of the space to the point (u, v) of σ_λ satisfies a Monge-Ampère equation whose coefficients depend on $E_\lambda, F_\lambda, G_\lambda$ and their derivatives as well as on $\rho_\lambda, \frac{\partial \rho_\lambda}{\partial u}$ and $\frac{\partial \rho_\lambda}{\partial v}$. The equation is elliptic because $K_\lambda > 0$. Take P as the center of the largest sphere inscribed to σ_λ . We want to prove: Let $0 \leq \lambda_1 < \lambda_2 < \dots$ be a sequence tending to λ^1 , such that $(ds_{\lambda_1})^2, (ds_{\lambda_2})^2, \dots$ can be embedded. Then $(ds_{\lambda^1})^2$ can be embedded. This fact is established as soon as we know that in the sequence $\rho_{\lambda_1}, \rho_{\lambda_2}, \dots$ of analytic functions of (u, v) there exists a subsequence converging to an analytic limit function $\rho_{\lambda^1}(u, v)$ which solves Darboux's equation for $\lambda = \lambda^1$. This fact follows immediately from Theorem 2 of my paper⁴ if the conditions of the theorem can be verified. They are:

i) $|\rho_{\lambda_1}|, |\rho_{\lambda_2}|, \dots$ are uniformly bounded. This is a consequence of Bonnet's theorem which states that the diameter of a closed convex surface is bounded from above by a number depending only on a (positive) lower bound of the Gaussian curvature.

ii) $\left|\frac{\partial \rho_{\lambda_1}}{\partial u}\right|, \left|\frac{\partial \rho_{\lambda_1}}{\partial v}\right|, \left|\frac{\partial \rho_{\lambda_2}}{\partial u}\right|, \dots$ are uniformly bounded. This follows from $(d\rho_\lambda)^2 \leq (ds_\lambda)^2$.

iii) The coefficient of $\frac{\partial^2 \rho_\lambda}{\partial u^2} \frac{\partial^2 \rho_\lambda}{\partial v^2} - \left(\frac{\partial^2 \rho_\lambda}{\partial u \partial v}\right)^2$ in Darboux's equation can be assumed to be $= 1$ while the other coefficients are bounded for complex values of $u, v, \rho_\lambda, \frac{\partial \rho_\lambda}{\partial u}, \frac{\partial \rho_\lambda}{\partial v}$ in a bounded neighborhood of their values under consideration. The contrary assumption is ruled out by a simple geometrical consideration showing that the radii of the maximum spheres inscribed in $\sigma_{\lambda_1}, \sigma_{\lambda_2}, \dots$ are bounded away from zero.

iiii) Elimination of ρ_λ . The independent variables (u, v) considered as functions of variables (α, β) which are characteristic with respect to the Monge-Ampère equation, satisfy two equations of the form

$$\begin{aligned} \frac{\partial^2 u}{\partial \alpha^2} + \frac{\partial^2 u}{\partial \beta^2} &= h_{1\lambda} \left[\left(\frac{\partial u}{\partial \alpha} \right)^2 + \left(\frac{\partial u}{\partial \beta} \right)^2 \right] + h_{2\lambda} \left(\frac{\partial u}{\partial \alpha} \frac{\partial v}{\partial \alpha} + \frac{\partial u}{\partial \beta} \frac{\partial v}{\partial \beta} \right) + \\ &\quad h_{3\lambda} \left[\left(\frac{\partial v}{\partial \alpha} \right)^2 + \left(\frac{\partial v}{\partial \beta} \right)^2 \right] + h_{4\lambda} \frac{\partial(u, v)}{\partial(\alpha, \beta)}, \\ \frac{\partial^2 v}{\partial \alpha^2} + \frac{\partial^2 v}{\partial \beta^2} &= k_{1\lambda} \left[\left(\frac{\partial u}{\partial \alpha} \right)^2 + \left(\frac{\partial u}{\partial \beta} \right)^2 \right] + \dots + k_{4\lambda} \frac{\partial(u, v)}{\partial(\alpha, \beta)}, \quad (1) \end{aligned}$$

where $h_{1\lambda}, h_{2\lambda}, \dots, k_{1\lambda}, k_{2\lambda}, \dots$ depend on (u, v) , but not explicitly on ρ_λ nor $\frac{\partial \rho_\lambda}{\partial u}$ nor $\frac{\partial \rho_\lambda}{\partial v}$. In our case $\alpha + i\beta$ and $\alpha - i\beta$ are parameters on the asymptotic lines and our equations (1) are identical with equations already found by Darboux.⁵

¹ Hermann Weyl, "Über die Bestimmung einer geschlossen konvexen Fläche durch ihr Linienelement." *Vierteljahrsschrift der naturf. Ges. Zürich*, 61, 40-72 (1915).

² I have been in possession of this proof for several years, cf. *Bull. Am. Math. Soc.*, 42, 824 (1936). The preparatory investigations of Monge-Ampère equations were undertaken mainly for this purpose and published in *Trans. Am. Math. Soc.*, 37, 417-434 (1935) and 41, 365-374 (1937).

³ G. Darboux, *Théorie des surfaces*, vol. 3, p. 260 (Paris, 1894).

⁴ Loc. cit., 41, 373 (1937); also *Bull. Am. Math. Soc.*, 42, 689-692 (1936).

⁵ Loc. cit., p. 290.

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

March 15, 1938

Number 3

Copyright 1938 by The National Academy of Sciences

*THE BAR "LOCUS" AND THE v^+ REACTION IN DROSOPHILA
MELANOGASTER*

BY ARTHUR G. STEINBERG AND MORTIMER ABRAMOWITZ

DEPARTMENT OF ZOOLOGY, COLUMBIA UNIVERSITY

Communicated February 5, 1938

Beadle and Ephrussi,² using the transplantation technique (Ephrussi and Beadle³), found that the formation of wild type eye pigmentation is dependent upon the presence of at least two substances, the so-called vermilion⁺ (v^+) and cinnabar⁺ (cn^+) substances. It was shown that while vermilion eyed flies were completely lacking in both these substances,¹ wild type flies not only possessed them but could supply them to implanted eyes. Thus, the only source of the v^+ and the cn^+ substances for an eye disc implanted into a vermilion host is the eye disc itself, while an eye disc implanted into a wild type host may secure these substances from the host as well. Beadle and Ephrussi reported that imaginal discs of the Bar eye of *Drosophila melanogaster*, when implanted into vermilion hosts, developed vermilion rather than + pigmentation, whereas imaginal discs of heterozygous Bar eyes developed + pigmentation when implanted into vermilion hosts. They offered further evidence to show that the failure of the homozygous Bar eye to form + pigmentation is not due to its smaller size. Beadle and Ephrussi suggested that the failure of the Bar eye to develop + pigmentation may be due to a retardation of certain reactions in the eye relative to the states of certain developmental reactions in other organs, and that this retardation is due to a recessive effect of Bar.

The experiments reported below are the first of a series designed to test further the above hypothesis in order to investigate this phase of the "Bar reaction." It is hoped that as a result some further light may be shed on the Bar reaction as a whole as well as on the method of pigment formation in the eye.

By means of the Ephrussi and Beadle transplantation technique the imaginal discs of the following Bar "alleles" (with wild type eye color) and their heterozygotes with + were implanted into vermilion hosts (with wild type eye size): B , BB , B' , $B'B'$ and B^4 (Bar, double Bar, infra Bar, double infra Bar and Bar⁴, respectively). In addition, eyes containing

B in combination with *m(B)*, a semi-dominant inhibitor of *B* located in the second chromosome (Steinberg unpublished), were implanted into vermilion hosts. Both donors and hosts were larvae about to pupate. All experiments were conducted at $25 \pm 1^\circ\text{C}$. In all except two cases the controls were implants of the same Bar genotype into wild type hosts (normal eye size and pigmentation), and the same Bar genotype in combination with vermilion implanted into vermilion hosts. The two cases in which this was not true are *B'B'/B'B'* and *B/B; m(B)/m(B)* (modified Bar). The controls for *B'B'/B'B'* were *BB/+* into *+* and *vBB/v* into vermilion. Those for *B/B; m(B)/m(B)* were *B'B'/+* into *+* and *vB'B'/v* into vermilion. This was done because the facet numbers of *B'B'/B'B'* and *BB/+* and of *B/B; m(B)/m(B)* and *B'B'/+* are alike or very nearly so. In all cases judgments were based on direct comparison of test and control implants; for example, Bar eyes implanted into vermilion hosts were compared at the same time to Bar eyes implanted into wild type hosts and to vermilion Bar eyes implanted into vermilion hosts (cf. table 1). Inasmuch as neither the sex of the donor nor that of the host had any effect on the phenotype of the implant, sex is not considered in the following report.

Before the data are discussed as a whole, it is necessary to call attention to the pigmentation of the homozygous and heterozygous Bar eyes, when implanted into vermilion hosts. As stated above, Beadle and Ephrussi² reported that *B/B* developed vermilion pigmentation, while *B/+* developed *+* pigmentation when implanted into vermilion hosts. Reference to table 1 will show that in our experiments *B* implanted into vermilion hosts was slightly but definitely darker than *vB* implanted into vermilion hosts, and that *B/+* implanted into vermilion hosts was considerably lighter than *B/+* implanted into *+* hosts. Since our experiments were conducted at the same temperature and with larvae of the same age as those used by Beadle and Ephrussi, we are at a loss to account for the differences between our results and theirs. The data in table 1 show that *B* anlagen implanted into vermilion hosts develop some *+* pigmentation and that *B/+* implanted into vermilion hosts is intermediate in pigmentation; it follows therefore that the effect of the Bar "locus" on the pigment reaction is not a simple recessive effect as Beadle and Ephrussi suggest.

The facet numbers given in table 1 are, in all except two cases (modified Bar and Bar⁴), taken from Sturtevant⁴ and are used as a standard to indicate the relative sizes of the eyes of the various donors. The Bar "alleles," when implanted into wild eyed hosts, retain the same size sequence as is shown by the non-implanted eyes. In this respect it is of interest to note that the imaginal discs of the various Bar "alleles," as seen in late third instar larvae, also fall into the same size sequence. The data point to some direct correlation between facet number and depth of pigmentation.

The correlation is, however, subject to two exceptions: 1. $B^iB^i/+$ which is approximately one-half as large as B^i/B^i is not only not lighter than B^i/B^i but is considerably darker, and 2. B^4 , which is exceeded in facet number only by $B^i/+$ and $+$, is lighter than $B^iB^i/+$, $B/+$ and B/B ; $m(B)/m(B)$, all of which are less than one-half as large as B^4 .

These discrepancies indicate that, although both the reduction in facet number and the partial or complete loss (cf. BB/BB table 1) of the ability to form the v^+ substance are due to changes at the Bar "locus," they are probably a result of two separate chains of reactions; i.e., the reaction chain resulting in the reduction in facet number is not the one involved in the pigment effect. If this is true, it should be possible to affect one of the reaction systems without affecting the other. It should be possible, for

TABLE 1

DATA ON THE VARIOUS EYE IMPLANTS

The hosts in all cases were v . v pigmentation = 1, $+$ pigmentation = 5 on an arbitrary scale. Values in brackets are those reported by Beadle and Ephrussi¹ in comparable experiments. In all except two cases ($B^iB^i/+$ and B/B ; $m(B)/m(B)$, see text) the controls were implants of the same Bar genotype into wild type hosts (wild type controls) and the same Bar genotype in combination with vermilion implanted into vermilion hosts (vermilion controls).

GENOTYPE	IMPLANT FACET NUMBER*	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
BB/BB	25	10	1.0
B^iB^i/B^iB^i	38	12	1.5—
$BB/+$	45	15	1.5—
B/B	68	18	1.5— (1.0)
$B^iB^i/+$	200	14	3.0
B/B ; $m(B)/m(B)$	200	9	3.0
B^i/B^i	348	13	2.0
$B/+$	358	7	3.0 (5.0)
B^4/B^4	?	8	2.5
$B^i/+$	716	19	5.0—
$+/+$	779	13	5.0—

* See text.

example, to cause a change in facet number resulting from a given genotype without causing any change in the pigment reaction and vice versa. Experiments are under way to test this hypothesis. An alternative but not as probable an explanation of the data is that B^4/B^4 and $B^iB^i/+$ may involve a qualitatively new situation as compared with the other genotypes, thus producing the observed discrepancy.

During the course of these experiments nineteen fragmented eyes were recovered from transplanations involving $+^vB^{\text{alleles}}$ eye discs implanted into vermilion hosts. Table 2 lists these cases showing the total number of fragmented eyes recovered and the number of these which did not develop

as much pigment as did the unfragmented eyes.⁵ In no case was a fragmented eye darker than an unfragmented eye. In the cases of $BB/+$ and B/B it is difficult to be certain that the fragments really were not lighter in pigmentation than the entire eyes, since the latter were already very close to vermilion. Consequently, they are not included in the following discussion.

Five of the fragmented eyes failed to develop the same pigmentation as the intact eyes. The fragmented eye recovered in the $B^i/+$ experiment was broken into four pieces. The two largest developed as much pigment as did the unfragmented eyes, the smallest fragment was lightest (vermilion or very close to vermilion), and the fourth fragment was inter

TABLE 2

DATA ON FRAGMENTED EYES RECOVERED FROM EXPERIMENTS IN WHICH THE DONORS WERE $+vB$ "alleles" AND THE HOSTS WERE v^b

IMPLANT GENOTYPE	FACET NO.*	PHENOTYPE OF INTACT EYE	NUMBER OF FRAGMENTED EYES	NUMBER FAILING TO DEVELOP SAME PIGMENT AS INTACT EYES*
$BB/+$	45	1.5—	1	0
B/B	68	1.5—	2	0
$B/B;m(B)/m(B)$	200	3.0	1	1
$B^iB^i/+$	200	3.0	4	1
$B/+$	358	3.0	1	0
$B^i/+$	716	5.0—	6	1
$+/+$	779	5.0—	4	2

* See text.

mediate in both size and pigmentation. Of the two fragmented eyes recovered in the $+/+$ experiment one consisted of six pieces and the other, of four. Nine of these ranged in size from smaller than B to B , all being close to vermilion in pigmentation. One fragment about the size of $B^iB^i/+$ was only slightly lighter than the unfragmented eyes. In the remaining two cases ($B^iB^i/+$ and $B/B;m(B)/m(B)$) the eyes were each broken into two pieces. In both cases the smaller fragment was vermilion, the larger the same as the intact eyes. In all of the above cases the smaller fragments were as light as or lighter than the larger ones.

Of the eleven fragmented eyes which developed the same amount of pigment as did the intact eyes, those arising in the $B^iB^i/+$, $B^i/+$ and $+/+$ experiments are of particular interest. Out of a total of fourteen fragmented eyes recovered in these three experiments, four failed to develop the "normal" pigmentation (one in each of the two former, two in the latter). In the ten remaining eyes many of the fragments, which did develop as much pigment as the unfragmented eyes, were as small as or smaller than those which failed to do so. This makes it seem highly

probable that the ability or lack of ability of a fragment to form the v^+ substance involves at least two factors, namely, the size of the fragment itself and the portion of the disc from which the fragment arises. Experiments are planned in which both the size and origin of the fragment will be controlled.

Summary.—The various Bar “alleles” and their heterozygotes with + were implanted into vermilion.

There is an apparent but not entirely consistent correlation between facet number and ability to form the v^+ substance, i.e., smaller eyes show less + pigmentation.

It is suggested that the facet reducing reaction system is not the same as the reaction system affecting pigment formation.

Evidence is offered which indicates that the ability of a fragment to form the v^+ substance is a function of the size of the fragment and of the portion of the disc from which the fragment arises.

The authors wish to acknowledge their indebtedness to Dr. Boris Ephrussi of the Institut de Biologie Physico-Chimique, with whom the senior author carried on preliminary work on these experiments at the Carnegie Institute of Washington, in Cold Spring Harbor, during the Summer of 1937, and to Professor L. C. Dunn of Columbia University for his many helpful suggestions.

¹ Beadle, G. W., *Genetics*, **22**, 587–611 (1937).

² Beadle, G. W., and Ephrussi, Boris, *Ibid.*, **21**, 225–247 (1936).

³ Ephrussi, Boris, and Beadle, G. W., *Amer. Nat.*, **70**, 218–225 (1936).

⁴ Sturtevant, A. H., *Genetics*, **10**, 117–147 (1925).

⁵ In experiments involving $+^vB^{(alleles)}$ eye discs implanted into + hosts 14 fragmented eyes were recovered. In all 14 cases the fragmented eyes developed the same pigmentation as the intact eyes.

SEX REACTION TYPES AND THEIR INTERRELATIONS IN *PARAMECIUM BURSARIA*. I

By H. S. JENNINGS

ZOÖLOGICAL LABORATORY, JOHNS HOPKINS UNIVERSITY

Communicated February 1, 1938

The discovery by Sonneborn¹ of two sex reaction types in a race of *Paramecium aurelia* opened a new chapter in the genetics of the Ciliata. The present study shows that similar reaction types occur in *Paramecium bursaria*, but that in number and interrelationships they differ greatly from the situation in the race of *Paramecium aurelia* studied by Sonneborn and by Kimball.²

In the work on the cytology of conjugation in *Paramecium bursaria*, in this laboratory, Dr. T. T. Chen observed that conjugating pairs could be obtained from mixtures of two of his cultures, but not from either culture separately. This formed the starting point of the present investigation. Two clones, designated *l* and *m* were obtained from the cultures of Chen, and the work was extended to many other stocks. The clones *l* and *m* had been collected from a pond at Alexandria, Virginia.

The mating reaction that occurs when two appropriate clones of *Paramecium bursaria* are mixed—such clones as *l* and *m*—is of the rapid agglutinative character described by Sonneborn¹ in *Paramecium aurelia*, and by Moewus³ for various flagellates. Within a few seconds after mixture the individuals have clotted together in small groups containing two to a dozen or more individuals. In strongly marked reactions these groups quickly coalesce into masses that contain hundreds of individuals and are visible to the naked eye. The animals remain clotted for a half hour to two hours; in the latter part of that period the clots begin to break up, and it is now found that many of the component individuals have become united in pairs. After about two hours only united pairs and single individuals may be found in the mixture.

The pairs are formed from one member of each of the two clones that were mixed. This is readily demonstrated by removal of the green color from individuals of one of the two clones. The usual strong green color of *Paramecium bursaria* is due to the presence in the body of many cells of a green alga. The color can be almost completely removed by inducing the animals to multiply rapidly in a rich nutritive medium, such as the algal-bacterial medium described by Raffel.⁴ The contained algae multiply much less rapidly than the Paramecia, so that the later generations contain but a few scattering alga cells which hardly color them at all. In this condition *Paramecium bursaria* is practically as colorless as *Paramecium aurelia*. When one of the two clones is thus made white, the pairs in the mixture are

found to consist always of one green individual and one white one. In the large clots also, green individuals are always in contact with white ones.

The reaction between two clones belonging to diverse reaction types occurs whenever the two clones are mixed, provided they are in the proper physiological condition. There is a period after conjugation, varying in length in different clones, in which the individuals will not mate. Further, mating does not occur when the individuals have recently been transferred to a rich nutritive medium and have grown very large and are rapidly dividing. The most favorable conditions for the mating reaction are furnished when, after such a period of rapid growth and division, the nutritive medium becomes poor and the animals are becoming thin and ceasing to divide. At such periods, if two clones of diverse type are mixed the clotting is strongly marked and many pairs are formed.

Different clones show marked differences in the readiness with which they are brought into the condition for clotting and conjugation. Some are refractory and can be induced to react only by bringing about the exact degree of nutritive decline that is most favorable to the mating reaction. Others react readily under all conditions except those of extreme plumpness and rapid fission resulting from rich nutritive conditions. The reaction occurs, however, only at certain periods of the day, diverse in different stocks, but excluding in all cases the evening hours, so far as observed. (These matters are to be dealt with separately.)

The clotting reaction above described occurs only when two clones of diverse sex reaction type are mixed. But in certain clones, perhaps in all, isolated pairing may occur between members of the same clone, if the clone is left for long periods in a condition of declining nutritive strength. If, for example, great numbers of individuals of a single clone are left for several days in a watch glass with a small amount of water, after some days isolated pairs may sometimes be found among them. The formation of such pairs is not accompanied by clotting, and is so rare that close study of a clone for months may yield no examples. Such exceptional pairs are left out of account here; they form no part of the usual picture of pairing. Their genetic consequences will be dealt with elsewhere.

In such clones as *l* and *m* we thus find two sex reaction types that are seemingly comparable to the plus and minus sex types of *Chlamydomonas* and other flagellates as described by Moewus,³ and to types I and II of *Paramecium aurelia* (Sonneborn 1).

The matter was investigated further (1) by allowing *l* and *m* to conjugate together, producing many exconjugant clones, the sex reactions of which were examined, and (2) by collecting from natural habitats many stocks and studying their sex reactions. Results of the former line of work are presented in this paper.

Sex Reaction Types of Clones Descended from Exconjugants of Type l by Type m.—A large number of pairs were obtained by matings between the clones *l* and *m*, representing the two reaction types. From the exconjugants of these pairs clones were obtained, and the sex type of each was tested by examining its reactions against the two parental types. The design was to obtain if possible inheritance ratios for the two diverse types.

This purpose meets a serious obstacle in the great mortality among the exconjugant lines. Most of the exconjugants or their descendants die. This occurs even in the most favorable nutritive media and other conditions in which non-conjugants flourish, none dying. A large proportion of the exconjugants die without dividing. Others divide, but produce weak offspring, often small and abnormal, which die after a few generations. In the experiments, 142 pairs were isolated from $l \times m$; these should yield 284 exconjugants, and as two lines were to be cultivated from each exconjugant, there were potentially 568 lines of descent. But out of these, only 26 formed clones that continued to multiply and so could be tested; thus but 4.6 per cent. And these included representatives of but 11 of the 142 pairs.

With so high a mortality the determination of inheritance ratios for the sex reaction types is not possible, but study of the 26 descendant clones nevertheless furnishes important information as to the sex reaction types and their nature.

The exconjugant lines were designated as follows. To each pair was given a number, and the two members of a pair were called *a* and *b*. From each member two lines of descent, derived from the first division of the exconjugant, were isolated; these were designated 1 and 2. Thus the pair 67 furnished the four lines of descent 67*a*1 and *a*2, 67*b*1 and *b*2. The 26 exconjugant lines were tested by mixing samples of them respectively with *l* and with *m*, to determine the sex type. As was expected, some reacted only with *l*, others only with *m*.

But the clone 67*a*1 was found to form pairs with both *l* and *m*, and on examination this was found to be the case with all the 4 clones descended from pair 67—*a*1, *a*2, *b*1 and *b*2. They reacted strongly, forming immediately large clots, and numerous later pairs, equally with *l* and with *m*.

It was at first suspected that such clones must have become differentiated into the two sex types, as *Paramecium aurelia* differentiates into two sex types at endomixis, according to Kimball.² But examination showed that this is not the case. Any single individual of 67*a*1 (or the other clones of 67) reacts with equal readiness with either *l* or *m*.

This is discovered as follows. A single individual of 67*a*1 is introduced with capillary pipette into a drop containing individuals of clone *l* only. The first individual of *l* with which it comes in contact clings to it and initiates the pairing reaction. While in this condition, before firm union, the pair are removed to a drop containing only individuals of *m*. In the transfer

the two become separated, since they have not yet firmly united. Each then provokes a strong reaction with *m*; in a few seconds two small clots are formed. In one of these individuals of *m* are clinging to *l*, in the other to 67*a*1. It is demonstrated that the single individual of 67*a*1 reacts with equal readiness with type *l* or type *m*.

		1				2								3										4								
		m	C	23b1	23b2	-	B	3a1	5b1	15b1	28b1	107a1	107a2	88a1	5b2	67a1	67a2	67b1	67b2	88a2	88b1	88b2	97a1	97a2	97b1	97b2	169a2	1b1	169a1	169b1	169b2	
1	m	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	C	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	23b1	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	23b2	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	l	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	B	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	3a1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	5b1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	15b1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	28b1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	107a1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	107a2	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	88a1	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
3	5b2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	67a1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	67a2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	67b1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	67b2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	88a2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	88b1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	88b2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	97a1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	97a2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	97b1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	97b2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
4	169a2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
	1b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
	169a1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
	169b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
	169b2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

TABLE 1
PARAMECIUM BURSARIA
Clones of the *l* - *m* group, including 26 clones derived from exconjugants of *l* × *m*.
Classified table of matings, showing the four reaction types.
+ Form clots and pairs when mixed.
- Do not clot and pair.

Also, if a single individual of 67*a*1 is allowed to multiply, and some of its descendants are tested with *l*, others with *m*, they are found to react immediately with both.

Thus we have, besides *l* and *m*, a third reaction type, which unites with both *l* and *m*. We are not dealing with simply two reaction types, as was at first assumed.

In the course of the tests a fourth reaction type was found, exemplified by the clone 1b1. This forms clots and pairs, not only with *l* and with *m*, but also with 67a1 and other members of its type.

To determine the exact situation, each of the 26 clones descended from *l* and *m* was tested with every other, as well as with the parental clones *l* and *m*. In addition, two other clones, *B* and *C*, were found to interact with members of this group; these were included in the tests. The testing of each of the 30 clones with each other one involves $\frac{1}{2}(30 \times 29)$ or 435 tests. In each test, 50 to 100 or more individuals of each of the two clones to be tested are placed together in 2 or 3 drops of water and observed for clotting and pairing.

The results of these 435 tests are shown in table 1. As the table shows, the 30 clones (26 derived from $l \times m$) fall into 4 diverse sex reaction types; there are indeed 4 types among the descendants of the two types $l \times m$.

The first of these is the *m* type, containing but four clones, including two descended from $l \times m$.

The second, the *l* type, includes nine clones; among them seven derived from the union of *l* and *m*.

The third, the 67a1 type, contains 13 clones, all derived from the union of *l* and *m*.

The fourth, the 1b1 type, contains 4 clones descended from $l \times m$. Thus the 26 exconjugant clones derived from the union of *l* and *m* fall into four reaction types in the proportions 2:7:13:4.

The four types have the peculiar relation to each other that the members of each one clot and conjugate with the members of all the other three. The relations for the four types are shown in table 2, in which the plus sign indicates clotting and pairing in the mixture indicated.

TABLE 2
THE FOUR SEX REACTION TYPES AND THEIR INTERACTIONS, IN THE *l* - *m* GROUP

	<i>l</i>	<i>m</i>	67a1	1b1
<i>l</i>	—	+	+	+
<i>m</i>	+	—	+	+
67a1	+	+	—	+
1b1	+	+	+	—

Certain points with regard to inheritance may be gleaned from table 1. From the union of two parent types, *l* and *m*, we obtain 4 types; two of which (*m* and 1b1) are less abundant than the other two. In some cases all four clones descended from a pair are of the same reaction type; this is the case in pairs 67 and 97. In other cases lines derived from the same pair,

and even from the same exconjugant of a pair, belong to different reaction types. This is the case for example with 5*b*1 and 5*b*2, with 88*a*1 and 88*a*2, with 169*a*1 and 169*a*2. A segregation of the sex types thus occurs in some cases at the first division after conjugation, as is true in *P. aurelia* (Sonneborn¹).

The clones produced from the exconjugants of *l* × *m* differ in many ways besides the differences in their sex types. There are characteristic differences in size, in form, in vigor and particularly in their readiness to react sexually. Some, like the parental clones, react quickly under almost any condition. In others, specially favorable conditions must be supplied, of the nature already mentioned, in order to make possible the characteristic reactions when they are placed with individuals of another type.

A discussion of the relation of these facts to the problem of sex differentiation is reserved until the sex reaction types of numerous stocks collected in nature have been presented.

¹ Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378-385 (1937).

² Kimball, R. F., *Ibid.*, **23**, 469-474 (1937).

³ Moewus, F., *Arch. f. Protistenk.*, **80**, 467-526 (1933).

⁴ Raffel, D., *Biol. Bull.*, **58**, 293-312 (1931).

SEX REACTION TYPES AND THEIR INTERRELATIONS IN *PARAMECIUM BURSARIA*. II. CLONES COLLECTED FROM NATURAL HABITATS

BY H. S. JENNINGS

ZOOLOGICAL LABORATORY, JOHNS HOPKINS UNIVERSITY

Communicated February 1, 1938

Twenty-seven clones of *Paramecium bursaria* were collected from diverse localities: four from near Alexandria, Virginia, twenty-three from the neighborhood of Baltimore, Maryland. After allowing the single individuals to multiply in the laboratory, the sex type of each clone was tested, originally by mixing with individuals of the two first-discovered types *l* and *m*, described in the foregoing paper.

The four Virginia clones, *l* and *m*, *B* and *C*, which all came from the same pond, were found to form but two reaction types, *B* being of the same type as *l*, *C* of the same type as *m*.

But unexpectedly, the clones from the region about Baltimore did not react with any of the Virginia types. As shown in the foregoing paper, two additional reaction types were developed from the two that originally occurred in the Virginia group. None of the 23 Maryland clones reacted with

any of the four reaction types of the Virginia group. In mixtures of any Maryland clone with any Virginia clone, there is no clotting or pairing.

The twenty-three Maryland clones were further tested by making mixtures of each clone with each other one, giving rise thus to 253 mixtures. Five of the Maryland clones did not react with any others. Repeated mixtures of these with each of the other clones were made, but no clotting or pairing occurred under any conditions.

		5						6						7				8	9
		AR	Ff	Fg	Fj	Fm	S	Fo	Fb	j	T	U	V	Fd	Fh	Fk	Fp	FI	Fo
5	AR	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
	Ff	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
	Fg	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
	Fj	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
	Fm	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
	S	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
6	Fo	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
	Fb	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
	j	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
	T	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
	U	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
	V	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	+	+
7	Fd	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+
	Fh	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+
	Fk	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+
	Fp	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+	+
8	FI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+
9	Fo	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—

TABLE 3
PARAMECIUM BURSARIA
Clones from natural habitats near Baltimore, Maryland, showing the five diverse sex reaction types (numbered 5 to 9).
+ Form clots and pairs when mixed.
— Do not clot and pair.

The other 18 Maryland clones reacted in some of the mixtures. The reactions fall into a system which is shown in table 3. Every clone conjugates with the members of certain other clones, and refuses to conjugate with those of certain others.

As table 3 shows, the 18 Maryland clones fall into five diverse reaction types, in addition to the four reaction types shown by the clones of the Virginia group. The Maryland types are numbered 5 to 9 in table 3. The reaction type 5 includes 6 clones, the members of which do not conjugate together, but do conjugate with the members of the four other types of the Maryland group. Type 6 again contains six clones, which do not interconjugate, but do conjugate with the members of the five other types of this group. The seventh type consists of four clones, while types 8 and 9 consist each of a single clone. Members of each type clot and conjugate with the members of every other type, but not with members of their own type.

Thus the nine diverse sex reaction types so far observed in *Paramecium bursaria* fall into two independent groups, the members of which do not conjugate with each other. The nine diverse types and their interrelations, with the fact that they fall into two groups, are shown in table 4.

The relations of the nine diverse reaction types to the localities from which they come are of interest. The four reaction types (numbers 1 to 4) which constitute the Virginia group all come from the same pond at Alexandria, Virginia, or are derived by descent from those that came from this pond. The five remaining types, constituting the Maryland group, come from two ponds near Baltimore. Type 5 consists of six clones from a small pond ("Falls Road Pond") a few miles north of Baltimore. Other clones from this pond are found in the other four types, 6 to 9. Type 6 contains four clones (*j*, *T*, *U*, *V*) from the central pond of the Botanical Garden of Johns Hopkins University, and in addition two clones from the Falls Road Pond. The remaining three reaction types are all composed of clones from the Falls Road Pond. Thus the Falls Road Pond contained members of all the five reaction types of the Maryland group.

In view of the two diverse reaction groups formed respectively by the clones from Virginia on the one hand, by those from Maryland on the other, examination of clones from other localities will be of interest; the author

	1	2	3	4	5	6	7	8	9
1	—	+	+	+	—	—	—	—	—
2	+	—	+	+	—	—	—	—	—
3	+	+	—	+	—	—	—	—	—
4	+	+	+	—	—	—	—	—	—
5	—	—	—	—	—	+	+	+	+
6	—	—	—	—	+	—	+	+	+
7	—	—	—	—	+	+	—	+	+
8	—	—	—	—	+	+	+	—	+
9	—	—	—	—	+	+	+	+	—

TABLE 4
PARAMECIUM BURSARIA

The nine sex reaction types thus far discovered, with their interactions, showing the two groups. Types 1 to 4 form the first or Virginia group, types 5 to 9 the second or Maryland group.

+ Form clots and pairs when mixed.

— Do not clot or pair.

expects to study such, as they can be obtained. The conditions thus far found suggest that there may be many local races, differing in sex reaction type, and not reacting with races from distant localities.

A deeper understanding of these types must wait upon a study, with adequate numbers, of their inheritance in crosses and in self-fertilization. Such a study is in progress.

What is the relation of the findings in this and the preceding paper to the differentiation of sexes? In contradistinction to the flagellates investigated by Moewus, such ciliates as *Paramecium* are diploid and each of the two mates furnishes a migratory pronucleus and a stationary pronucleus, the former playing the part of a male gamete, the latter the part of a female gamete. There is thus a reciprocal fertilization, and each of the two mates gives rise to a line of descendants. The two mates are thus equivalent in these respects; each is comparable to a hermaphrodite rather than to a male or female. Nevertheless the two mates are differentiated in relation to the mating reaction, and the studies thus far made point to an interesting series of steps leading toward the development of two definite types comparable to two sexes. The observations on *Paramecium bursaria* here set forth suggest nothing of the sort. They show the existence of many diverse types, the members of any one of which do not conjugate together (under ordinary conditions), but do conjugate with members of various other types. But in *Paramecium aurelia*, as shown by Sonneborn and by Kimball, in a given race there are two and only two reaction types, and any single individual may, by endomixis, produce these two but no others. Yet each type still plays in other respects the part of a hermaphrodite. In the Vorticellidae the differentiation has gone farther; the two types are morphologically diverse, and one plays essentially the rôle of a female, the other that of a male. Seemingly two definite sexes have arisen by gradual specialization among an originally large number of diverse reaction types.

THE RÔLE OF THE MELANOPHORE-DISPERSING HORMONE OF
THE PITUITARY IN THE COLOR CHANGES OF THE CATFISH

BY CLINTON M. OSBORN

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated February 3, 1938

Until recent years, color changes in the teleosts were thought to be solely under nervous control. It is the aim of this preliminary paper to report experiments which indicate that in the chromatic behavior of the catfish, *Ameiurus nebulosus*, the secretion of the intermediate portion of the pituitary is the most significant factor in the production of the dark phase of body coloration.

Catfishes between 6 and 8 inches long were collected from nature and used soon after their arrival at the laboratory. All experiments were carried out at 12°C. in slowly running water. Stock animals were kept under identical conditions. Standard black or white backgrounds illuminated by two 100-watt frosted Mazda lamps placed a foot apart and 12 to 15 inches above the aquaria were used.

Every "melanophore diameter" in this paper represents an average of the measurements of at least 25 consecutive dermal melanophores from corresponding areas along the middle ray of the tail fin.

Observations.—A. Background Adaptation of Hypophysectomized Catfishes. Fifty-four hypophysectomized catfishes were used to study the rate of the disappearance of the melanophore-dispersing principle from the circulation of the animal. Immediately after the operation these animals were divided into three groups and placed with unoperated controls on black, intermediate and white backgrounds, respectively. Observations which were frequently recorded may be summarized as follows:

1. The responses of normal and hypophysectomized animals to a *white* background were identical. Both groups paled at a normal rate (Parker '34) and remained in that state as long as the same experimental conditions were maintained (10 days).

2. Freshly hypophysectomized and control catfishes adapted equally well to an *intermediate* background. During the first 8 or 10 hours following the operation the experimental animals were indistinguishable from controls. After that, however, the hypophysectomized fishes showed gradual paling which seemed to reach an end-point about 3 days after the operation. Although the pallor of the experimental animals made them distinguishable from the normal ones they were not much lighter. The melanophores of controls in this experiment measured 85 to 100 μ , while those of the paler hypophysectomized fishes were about 70 μ in diameter.

3. Freshly hypophysectomized catfishes darkened on a *black* back-

ground as effectively as controls but after 10 to 12 hours pallor gradually set in reaching its maximum in 80 to 90 hours. Subsequently the operated fishes exhibited a pronounced contrast to normal black-adapted animals. The melanophore diameter of such black-adapted animals was 125 to 130 μ . The operated black-adapted fishes possessed melanophores about 70 μ in diameter, a size presenting comparative pallor but still 20 to 25 μ larger than completely white-adapted melanophores, which average 45 to 50 μ .

Any of the experimental animals described in paragraphs 2 and 3 will lighten when placed on a white background so as to become indistinguishable from white-adapted controls.

B. Tests for the Presence of the Dispersing Principle in the Blood of Recently Hypophysectomized Catfishes. Because the above experiments suggested that a functional amount of dispersing principle apparently remains in the circulation for 3 or 4 days after hypophysectomy, samples of blood (centrifuged free from cellular content) were taken from catfishes at regular intervals during this time. All of the fluid obtainable from a single catfish was tested by subcutaneous injections into a black-adapted catfish which had been hypophysectomized 10 days. Although this is a crude and rather insensitive test, it indicated that at sixteen hours after hypophysectomy there is present in the circulation sufficient dispersing substance to darken a test animal markedly.

If adrenalin (just enough to produce pallor in a black fish, Bray '18) is injected at the time of hypophysectomy into animals placed on a black background they will pale rapidly. However, the paling effect of the adrenalin soon wears off and the animal adapts completely to the black background. Subsequently pallor, due to disappearance of the dispersing factor of the pituitary, sets in and becomes maximum in 3 or 4 days as previously stated. The adrenalin test made several hours after hypophysectomy indicates that some dispersing substance from the pituitary is available to influence the melanophores about 70 hours after hypophysectomy.

C. Color Reactions of Hypophysectomized Catfishes to Darkness. When catfishes which had been hypophysectomized several days were placed with controls in darkness two interesting observations were recorded. (1) All the operated animals paled to a shade lighter than intermediate but somewhat darker than complete white-adaptation. Control fishes became intermediate in color. (2) Control animals were not all of the same shade but showed considerable variation about intermediate. The operated catfishes were remarkably homogeneous in their characteristic paler shade. The hypophysectomized catfishes when blinded still retain this pale shade in darkness.

D. Responses of Hypophysectomized, Blinded Catfishes to Illuminated

Backgrounds. When a normal catfish in an illuminated environment is blinded, it darkens rapidly to a coal black (Parker '34, Abramowitz '36). This reaction is pronounced (melanophore diameter 130 to 140 μ), and is not modified by background, but is reversed to complete pallor by darkness (absence of light). Adrenalin injection will also lighten an animal darkened by blinding.

It therefore seems significant that when a catfish, which has been hypophysectomized and kept on a *black* background 4 or 5 days, is blinded, it does not darken. However, if the animal has been kept on a *white* background for the same length of time since hypophysectomy, blinding results in slight darkening to a shade equal to a black-adapted hypophysectomized animal (melanophore diameter about 70 μ).

If blinding precedes hypophysectomy the expected results obtain. A fish made coal black by blinding will become pale following hypophysectomy. This pallor never exceeds the condition typical of black-adapted hypophysectomized fishes. Ways of lightening such an animal more completely are: (1) placing in total darkness (as noted under C), (2) by adrenalin injection or (3) electrical stimulation. Because the melanophore diameter (130 to 140 μ) of blinded catfishes is reduced to 70 μ by pituitary removal, it appears reasonable to conclude that the dispersing material of the pituitary is responsible for a major part in the darkening process in catfish color change.

If the coal black reaction in blinded animals is allowed to persist more than a few days before hypophysectomy, pigment may accumulate (Odiorne '37) in sufficient quantities to mask the pallor caused by hypophysectomy. However, pigment degeneration will finally take place in the hypophysectomized fish and the color behavior previously described will obtain.

E. Influence of the Injection of Catfish Pituitary on Color Behavior. The foregoing experiments were checked by injections of a suspension of finely minced catfish pituitaries into hypophysectomized fishes. The glands were taken from animals adapted to an intermediate background. It is hoped that the pituitaries of catfishes from various environmental conditions of background and illumination may be assayed for melanophore dispersing potency in future experiments. However, in this series of injections, dosages of from 1 to 4 pituitaries equivalent were administered subcutaneously into catfishes which had been hypophysectomized at least one week. The results of the injections may be summarized thus:

1. The melanophore diameters of black-adapted hypophysectomized fishes increased from about 70 μ to 125 μ .

2. Blinded, hypophysectomized catfishes under illumination darkened to coal black typical of illuminated, blinded animals. The melanophores enlarged somewhat more than noted in paragraph 1.

3. Hypophysectomized animals in darkness darkened markedly. This is probably not the normal physiology of the animal because the normal intact catfish pales in darkness to intermediate or lighter. This darkening is probably due to the sudden introduction into the circulation of abnormally large quantities of the dispersing substance which may be in such abundance that concentrating nerves are unable to over-balance its effect.

4. Any normal or hypophysectomized catfish in an illuminated environment may be darkened by pituitary injection as has been shown by Odiorne '33 and Parker '34. That the darkening will occur even if the animal is on a white background may be explained as in paragraph 3.

The effects of all the above injections are but temporary and vary in length with the dosage given. The largest injections lasted only about 4 days. An interesting aspect of injection experiments of this type is that experimental deficiencies may be cancelled out by substitution therapy.

Discussion.—Parker ('34) concluded that catfishes darkened by the combined action of dispersing nerves and a dispersing neurohumor from the pituitary which he believed to be weak in action. Abramowitz ('36) confirmed Parker's work and also stated that denervated cells do not readily attain a condition of maximum dispersion in black-adapted hypophysectomized animals.

That the dispersing substance of the catfish pituitary has hitherto been considered weak and insignificant in normal color change may be traced to the fact that the animals were not studied long enough after hypophysectomy (Parker's animals lived only 2 days). This same criticism has been voiced by Veil ('37) who believes that the rôle of the pituitary's dispersing agent is as significant in the catfish as it has been considered in the elasmobranchs by Lundstrom and Bard ('32).

It seems clear that the dark phase of the catfish is caused by more than one factor. The dispersing agent of the pituitary apparently causes about two-thirds of the darkening but the remaining third which occurs in the absence of the pituitary must be explained by other mechanisms. Parker ('34) believes that dispersing nerve fibers are the chief dispersing agents. Abramowitz ('36) and Wyckes (personal communication) are of the opinion that unstimulated melanophores assume a "resting" condition of partial dispersion.

Summary.—The experiments in this preliminary paper indicate that the pituitary of the catfish secretes a melanophore-dispersing agent which is the most important single darkening factor in its chromatic system. Tests show that functional amounts of the dispersing material remain in the circulation of the catfish until about 70 hours after hypophysectomy (at 12°C.). Hypophysectomy does not annihilate color change but limits the range of shades which the animal may assume. A normal catfish may white-adapt (with melanophore diameter 45 μ to 50 μ) or may black-adapt

(with melanophores dispersed to $125\ \mu$ to $130\ \mu$). A blinded fish becomes coal black (melanophore diameter $130\ \mu$ to $140\ \mu$) on any illuminated background. When the pituitary's dispersing substance is absent from the circulation, blinding or black background (strongest dispersing stimuli) are incapable of dispersing the melanophores to a diameter greater than $70\ \mu$ to $75\ \mu$.

Abramowitz, A. A., "Physiology of the Melanophore System in the Catfish, *Ameiurus*." *Biol. Bull.*, **71**, 259-281 (1936).

Bray, A. W. L., "The Reactions of the Melanophores of *Ameiurus* to Light and to Adrenalin." *Proc. Nat. Acad. Sci.*, **3**, 58-60 (1918).

Lundstrom, H. M., and P. Bard, "Hypophyseal Control of Cutaneous Pigmentation in an Elasmobranch Fish." *Biol. Bull.*, **62**, 1-9 (1932).

Odiorne, J. M., "The Effects of the Pituitary Hormones on the Melanophores of Fishes." *Proc. Nat. Acad. Sci.*, **19**, 745-754 (1933); "Morphological Color Changes in Fishes." *Jour. Exptl. Zool.*, **76**, 441-465 (1937).

Parker, G. H., "Color Changes in the Catfish, *Ameiurus*, in Relation to Neurohumors." *Jour. Exptl. Zool.*, **69**, 199-233 (1934).

Veil, C., "Hypophysectomie et changement de couleur chez le poisson chat." *Comptes Rendus Acad. Sci.*, **124**, 111-113 (1937).

ON THE DUPLEXITY THEORY OF VISUAL RESPONSE IN VERTEBRATES

BY W. J. CROZIER, ERNST WOLF AND GERTRUD ZERRAHN-WOLF

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated February 8, 1938

The conception that the visual performance of vertebrates in general involves the separable functioning of two morphologically distinct kinds of retinal sensory elements arose from the suggestion of Schultze (1866). Observations have tended on the whole to support the proposition that retinal "rods" are concerned with visual response at lower illuminations, "cones" with excitation at higher intensities of light,¹ as based upon correlations between retinal histology and the ethology of the animals concerned, their nocturnal or diurnal activities and other features of the illumination of their habitats during their active movements.² There are serious difficulties, however, in the way of the application of histological criteria for "rod" and "cone," even in a single retina, and of considering them as invariable types.² On the other hand the phenomena of their photomechanical and otherwise induced movements³ and their "spontaneous" changes⁴ frequently serve to separate the sensory cells of the vertebrate retina into two physiologically

distinguishable categories, corresponding to the histological types labelled "rods" and "cones," respectively.

Of a logically different order are the kinds of evidence obtainable from measurements of the visual performance of vertebrates. Among fishes⁵ and amphibians⁶ thus far examined, and in man,⁷ having both rods and cones, the relation between flash-frequency (F) and critical illumination ($\log I$) for response to flicker is expressed as a double sigmoid curve (Fig. 1), a smaller one at lower intensities succeeded by a larger one at higher intensities. On the more or less general grounds already indicated, supported by the comparable evidence derived from the properties of diverse human visual functions,⁸ these two portions of the composite graph, signifying the activities of two groups of excitation elements, have been labelled (for convenience and consistency in reference)^{5,6,7} as respectively determined by the activities of rods and cones.

The identification would always remain defective, however, without some more direct demonstration. The occurrence of "double cones" in lower vertebrates is an example of the kind of complication which arises.⁶ The functional proof of the existence of two chief populations of sensory elements does not necessitate that these are to be severally identified with "rods" and "cones." Nor can the comparison of the visual function contours provided by various animals with the numbers and proportions of the rods and cones apparent in their retinas really settle the answer in a satisfactory way. The evidence thus obtained, while certainly consistent with the requirements of the duplicity doctrine, does not establish its inevitability.

There is required for a sufficient test the quantitative description of the visual performance of vertebrates possessing only "cones," or only "rods," as structurally defined. It is true that the curves for visual functions of arthropods established in this way show no such separation into two parts as is indicated by the graphs in figure 1, and that one has no evidence of their involving two or more distinct sorts of receptor units,⁹ but this evidence is not decisive. For another additional reason, it was important for our purpose to investigate the visual behavior of vertebrates exhibiting only one structural type of retinal element. The "composite" graphs of the vertebrate flicker contour have been separated into two portions¹⁰ by fitting to the ascending parts two log-probability integrals. Reasons for assuming such a function, rather than one of the photostationary equilibrium type for example, are discussed elsewhere.¹¹ Such separations have involved extrapolation of the "cone" curve over some little distance toward the $\log I$ axis. If one were to make suitable measurements with a rod-free vertebrate, which seems thus far not to have been done, it should be possible to discover if (1) the absence of "rods" brings with it the absence of the lower section of the typically composite graph, and (2) if the complete curve is in

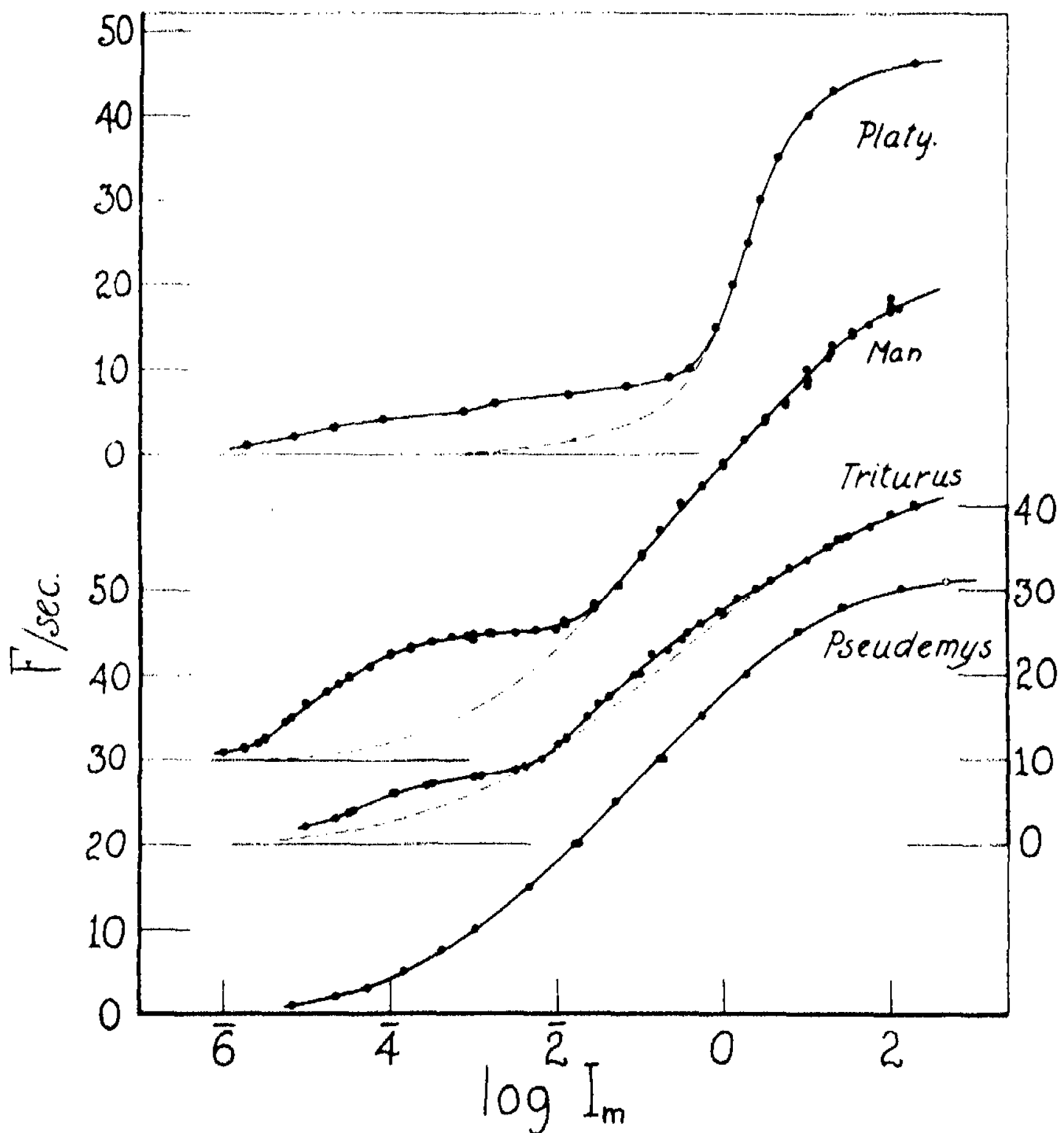


FIGURE 1

The relation between flash-frequency F and mean critical intensity for response to flicker, as obtained for several vertebrates, with a flash cycle in which the proportion of light-time to dark-time = 1. The curves for the fish *Platypoecilus*,^{7a} the newt *Triturus*,⁶ and the turtle *Pseudemys scripta* are based upon 30 observations at each point (3 on each of the same 10 individuals at each point, for each curve), at 21.5°; the curve for "man" gives averages of 10 observations for each point with one individual.⁹ (The curve drawn for *Pseudemys* is a probability integral; the slight departures are due to the fact that the 10 individuals whose curves have been averaged do not form a truly homogeneous group; a better fit is obtained for the individuals taken separately. The "hump" on the *Triturus* curve is due to another kind of complication.⁶ For the 3 upper curves the fitted probability integral has been extrapolated toward the $\log I$ axis; the difference between the observations and the extrapolated curve is attributed to the activation of rods, which beyond a certain intensity is no longer effective.)

fact adequately fitted by a log-probability integral over its whole extent. Experiments with a cone-free form would complete the formal argument, and provide certain tests of the "difference curves"^{6,10} in the case of the flicker-response contour.

If the quantitatively separable portions of curves of visual functions are to be allocated respectively to the functional activities of rods and cones, a modification of the customary argument concerning the duplicity theory is required. The comparison of flicker-reaction contours in different vertebrates makes it clear that a separation into "rod" and "cone" categories is scarcely significant in terms of intensity thresholds alone. The primary question is whether the data indicate the presence of one homogeneous population of sensory effects, or of more than one such population. Visible structural properties of the peripheral elements may be of only slight meaning for this matter, even when unambiguous.

As in other cases of significant description of functional properties, the whole course of the property as a function of its inciting variable must be determined. Thus when the tentatively labelled "rod" contribution to the flicker curve of a particular animal is small, the spread of the "cone" portion is likely to be great and its slope low.^{5,6,7} On the other hand, the converse relationship is not at all valid. It could not be said, in view of such facts, that a "rod"-poor retina is necessarily incapable of providing a receptor field for visual functioning at intensities so low as are possible with an animal well provided with rods. This curiously anti-"teleological" aspect of the general situation, which is emphasized by some additional observations, is related to the fact that the characteristic quantitative properties of "rod" and "cone" populations may be determined in separate and distinct ways by hereditary processes in a single type of animal.⁵ Facts of the latter kind, of course, give an additional proof of the existence of two kinds of elements active in visual response, but do not prove that they are respectively due to "rods" and to "cones." The significant labels, which avoid possibly irrelevant reference to histological criteria, could be perhaps *photopic* and *scotopic* populations of sensory effects, when these are distinguishable; if only one such is to be detected in a given animal, it is to be characterized by its measurable parameters.

The majority at least of diurnal turtles, like certain other reptiles, are said to be remarkably free from rod visual cells.¹² We have utilized the reaction of young individuals of *Pseudemys scripta* to visual flicker, as observable in the apparatus we have employed for work with insects,⁹ fishes,⁶ amphibians⁸ and man.⁷ The details of the procedure are described elsewhere, together with certain further considerations arising from the data. Examination of sections of the retina of these animals shows the absence of identifiable rods. If such cells are present they are very few indeed.

The flicker-response curve in figure 1 was obtained from *Pseudemys*,

using a flash-cycle with equally long light and dark intervals, at 21°5. It shows no trace of the composite character apparent in the usual flicker curve for a vertebrate. It is accurately described over its whole determinable extent by a single log-probability integral, which is the curve adjusted to the observations in figure 1.

The slope of the curve for *Pseudemys* is much lower than that obtained under the same conditions from various teleosts.⁵ The intensity range covered is however as great, and the maximum to which it rises is of the general order encountered in all vertebrates. That in the absence of histologically identifiable retinal rods a smooth probability integral is obtained for the whole relation of $\log I$ to F is a fairly decisive argument for the duplicity theory and for its interpretation of the compound character of the corresponding curves obtained with other forms. It also justifies the procedure we have employed for the analytical separation of the cone- and rod-parts of the typically composite flicker curve^{5,6,7} on the assumption that the contribution for which cone excitation is responsible should be, if separately visible over its lowermost portion, a complete probability integral in $\log I$.

Summary.—The absence of rods from the retina of the turtle *Pseudemys scripta* is correlated with the absence of a "rod" portion in the curve for response to visual flicker. This gives a proof for the validity of the essence of the duplexity doctrine. It permits the logical correlation of the occurrence of rods in diverse retinas with the presence of a presumptive rod contribution to the measured contours of visual performance. For this turtle the *flicker frequency* vs. *log critical intensity* curve is a smooth probability integral over its whole determinable range; this justifies the method of analytical dissection which has been employed with the composite F - $\log I$ curves of other vertebrates.

¹ Cf. v. Kries, J., *Handb. norm. path. Physiol.*, 12, 1, ii, 678–707 (1929).

Arey, L. B., *Special Cytology* (ed. E. V. Cowdry), edn. 2, 3, 1213–1304 (1932).

Granit, R., *Die Elektrophysiologie der Netzhaut . . .*, Copenhagen, 98 pp. (1936).

Hecht, S., *Physiol. Rev.*, 17, 239 (1937).

² Cf. Menner, E., *Zeits. vergl. Physiol.*, 8, 761 (1928).

Verrier, M.-L., *Bull. Biol. Fr. Belg.*, Suppl. 20, 140 pp. (1935); *Ibid.*, 71, 238 (1937).

³ E.g., Laurens, H., and Detwiler, S. R., *Jour. Exp. Zool.*, 32, 207 (1921).

⁴ Welsh, J. H., and Osborn, C. M., *Jour. Comp. Neurol.*, 66, 349 (1937).

⁵ Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *J. Gen. Physiol.*, 20, 211 (1936–37); *Ibid.*, 21, 17 (1937–38).

⁶ In course of publication: data on the Newt *Triturus*.

⁷ Cf. Hecht, S., Schlaer, S., and Smith, E. L., *Cold Spr. Harb. Symp. Quant. Biol.*, 3, 237 (1935).

^{7a} Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, 21, 203 (1937–38).

⁸ Hecht, S., *Physiol. Rev.*, 17, 239 (1937).

⁹ Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, 20, 363 (1936–37); *Ibid.*, 21, 223 (1937–38).

¹⁰ Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23**, 71 (1937).

Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, 516 (1937).

¹¹ *Journ. Gen. Physiol.*, **20**, 393, 411 (1936-37); and subsequent reports on the effects of alterations of the flash cycle.

¹² Detwiler, S. R., *Jour. Exp. Zool.*, **20**, 165 (1916).

Detwiler, S. R., and Laurens, H., *Jour. Comp. Neurol.*, **32**, 347 (1920).

Detwiler, S. R., *Jour. Exp. Zool.*, **37**, 89 (1923).

Walls, G. L., *Amer. Jour. Ophthalmol.*, **17**, 892 (1934).

Verrier, M.-L., *Biol. Bull. Fr. Belg.*, **71**, 238 (1937).

ON THE LAW FOR MINIMAL DISCRIMINATION OF INTENSITIES. III

BY W. J. CROZIER AND A. H. HOLWAY

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated February 8, 1938

1. The conception has been advanced that in properly designed measurements of a biological property the mean observed variation is due to the determinate variability of the performance of the organism,¹ rather than to experimental "error." This has a number of general consequences for the interpretation of such measurements, and is a significant factor in the choice of an explanatory mechanism. For example, response to visual flicker is a function of flash-frequency F and flash-intensity I ; and it is found that

$$\sigma_F = k\sigma_I \cdot (dF/dI), \quad (1)$$

where σ_F is the mean S. D. of the measurements of critical F at fixed values of I , σ_I that for the measured values of I at fixed levels of F .² Equation (1) may be derived on the assumption that the eventuation of the observed response of the reacting organism includes and evidences a variability which is primarily responsible for the measured σ_I and σ_F .

In general, a uniformly maintained end-point of reaction establishes the critical relationship between measurable variables A and B concerned in the evocation of response. The function connecting A and B exists in the form of a band defining the probability that repeated measurements of A as a function of B or the reverse, under similar conditions, will fall inside the band rather than outside it. An equation of the form of (1) then defines the nature of mean σ_A in terms of mean σ_B , and *vice versa*. The laws of the variation for A and for B will not commonly be of the same type.

2. It is important that the validity of equation (1) should be tested for a case in which the experimental manipulations are of the same kind when either A or B is the independent variable. This is most easily done with

measurements of visual intensity discrimination. With the discriminometer described elsewhere³ a circular light image of intensity I (visual angle

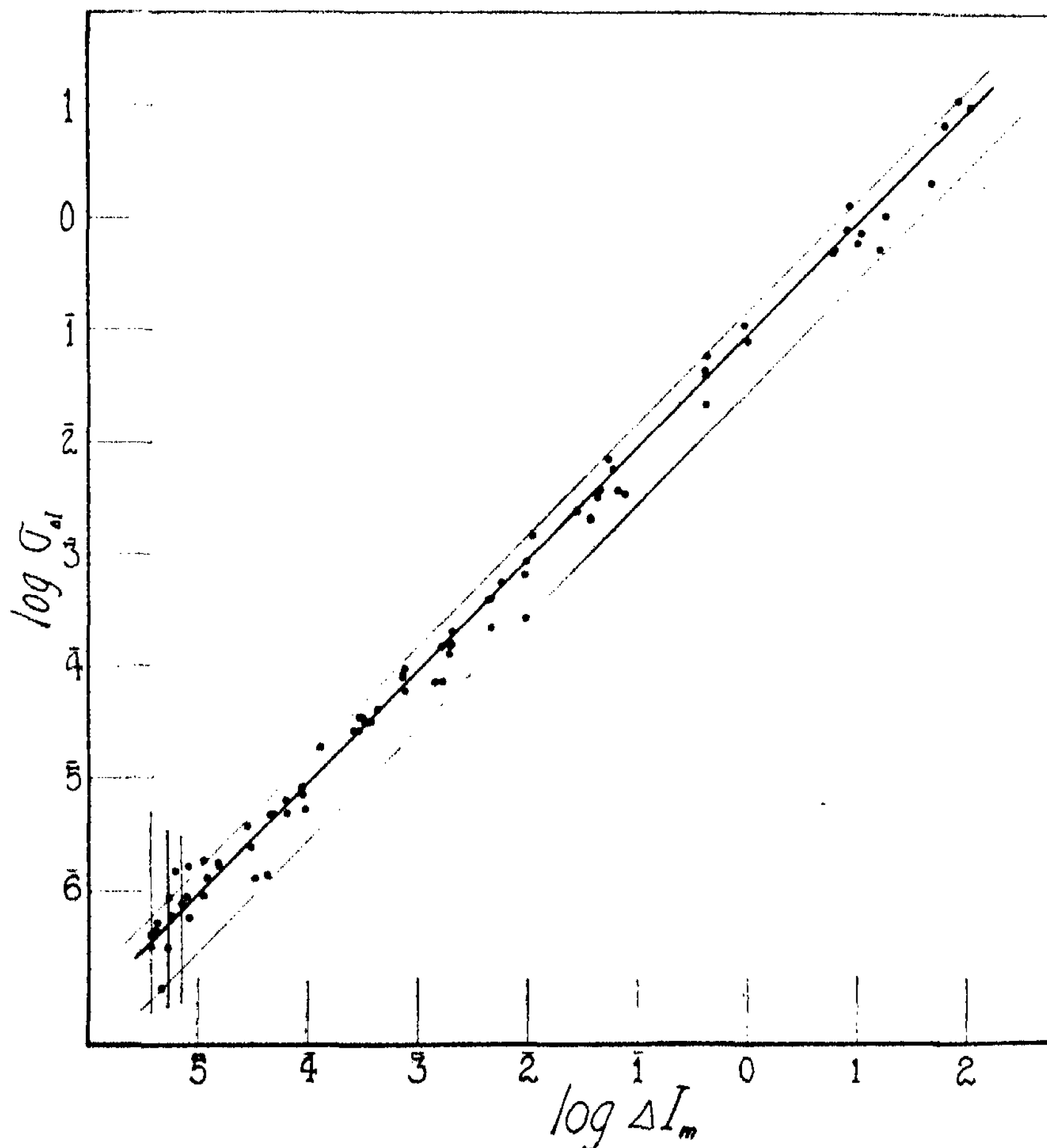


FIGURE 1

Data from measurements (one series) with ΔI exposed as a flash (0.20 sec.); I_1 fixed and continuously exposed. Each point is a mean of 5 observations. The law is the same as for cases in which ΔI is obtained by continuous exposure: $\sigma_{\Delta I} = k \Delta I$. This series is homogeneous, and the central line halfway between the extremes of $\sigma_{\Delta I}$ divides the field into equal numbers of points. The mean position of the stimulus-threshold, with its scatter ($\Delta I \approx \sigma_{\Delta I}$), is indicated by the vertical lines.

38°) is centered at the fovea on the retina. A second circular image (visual angle 16°) is added concentrically with the first. The larger area is then of intensity I_1 , the smaller I_2 and $I_2 - I_1 = \Delta I$, where ΔI is in this case the

added intensity¹ required to evoke the end-point response. I_1 may be fixed, and ΔI can then be *increased* until difference between I_1 and I_2 is detected. However in the reverse experiment the intensity of the 16° image can be fixed and I_1 then *lowered* until the difference between I_1 and I_2 is just detected. Since ΔI is added to I_1 to give I_2 we then have $I_2 - I_1 = \Delta I$. The instrumental manipulations producing adjustment of I_1 and of ΔI , respectively, are of the same kind in the two cases.³

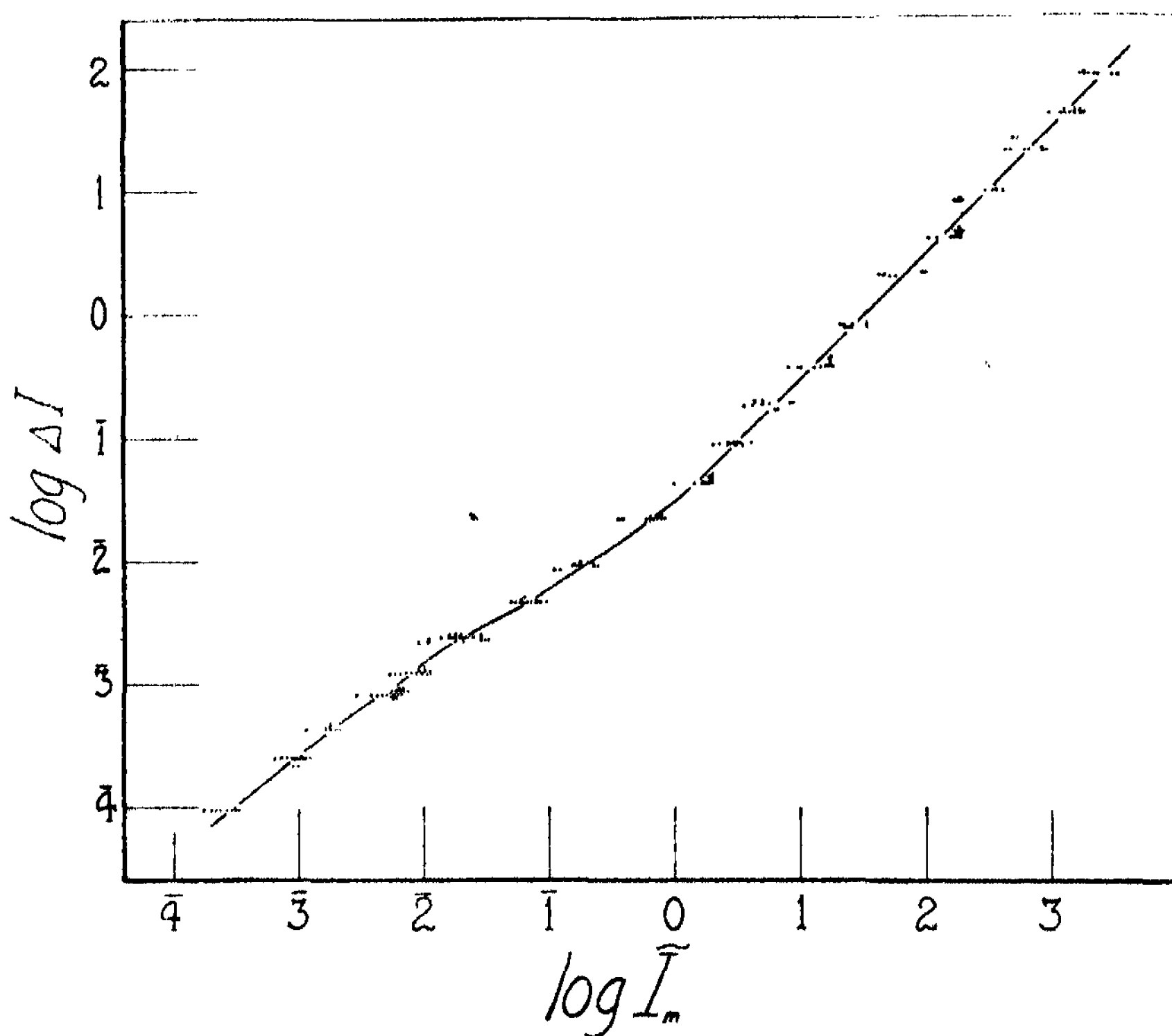


FIGURE 2

Log ΔI vs. log I . Solid dots, $(I_1)_m$ with $n = 5$, for fixed ΔI ; the vertical span is constant, which means that $\sigma_{\Delta I} / \Delta I$ is invariant. At any level of ΔI the central line gives log $(I_1)_m$.

The problem now is to predict the form of the variation of I_1 , as a function of mean I_1 when ΔI is fixed, from knowledge about $\sigma \Delta I$ when I is fixed⁴ on the assumption that the variation is due to the organism.

From (1) we have $\sigma_I = \sigma_{\Delta I} (dI/d\Delta I)$; dividing through by I ,

$$\sigma_I / I = \sigma_{\Delta I} (d \log I / d \Delta I);$$

we know experimentally⁵ that

$$(\sigma_{\Delta I})_m = k (\Delta I)_m,$$

hence

$$\sigma_I / I = K (d \log I / d \log \Delta I). \quad (2)$$

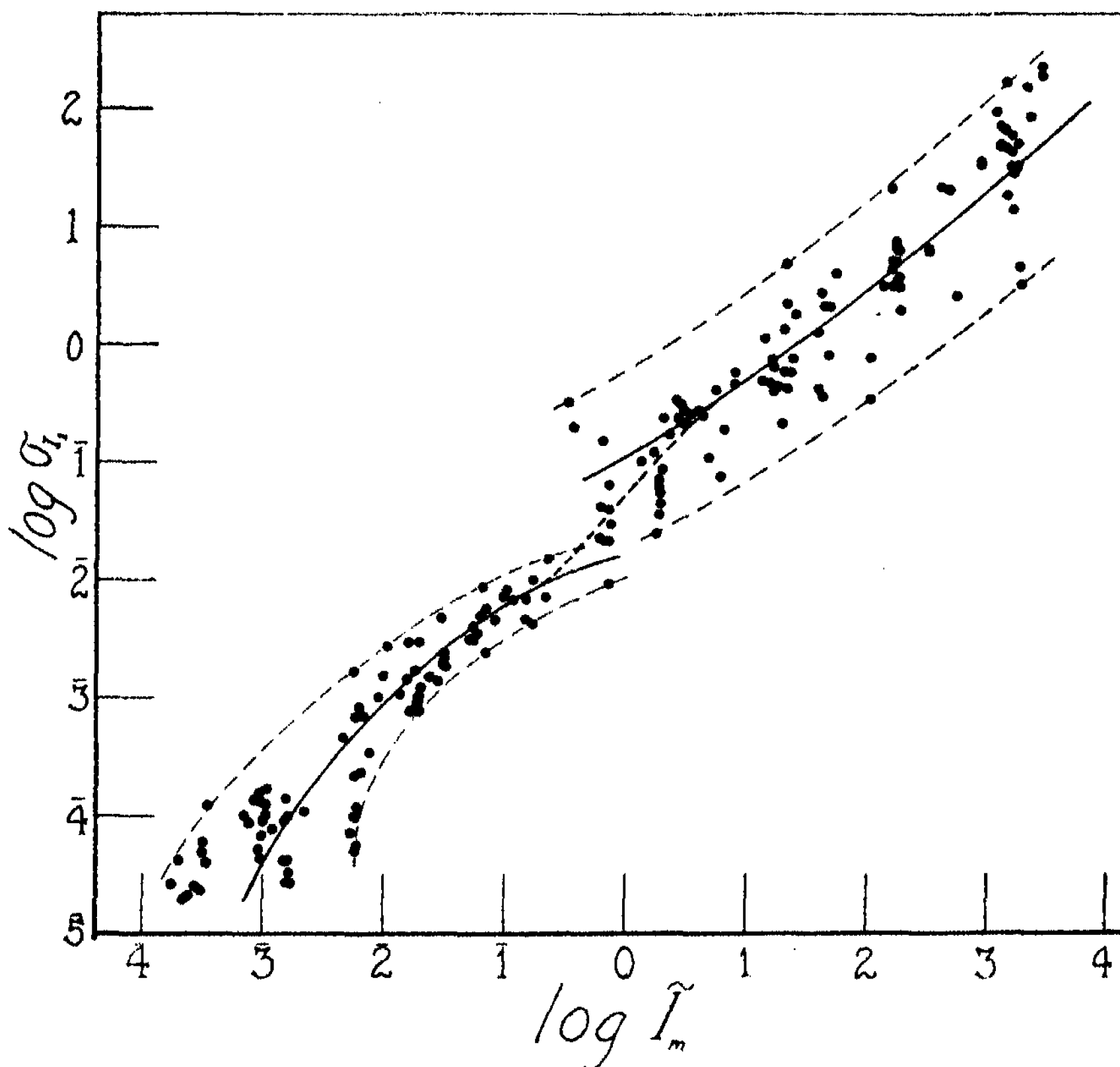


FIGURE 3

Log σ_I vs. log \bar{I}_1 . The graph is in two sections, the junction corresponding to the level at which discrimination becomes exclusively a "cone" function. The variation law is quite different from that for $\sigma_{\Delta I}$ (Fig. 1). The central line divides the plotted points equally; it does not give arithmetic division of the σ_I span (nor of the I_1 span), because five series of observations have been grouped.⁶

3. In the experiment devised to test this formulation the procedure was to present the adjusted intensity (\bar{I}_1 or $\Delta \bar{I}$) in a succession of 0.20 sec. flashes, with decrease (\bar{I}_1) or increase ($\Delta \bar{I}$) of intensity for the successive flashes until a difference between I_2 and I_1 was signaled as detected.⁶ Sets of 5 such readings were averaged for each fixed value of ΔI or of I , and σ_I computed for each set. Figure 1 shows that $(\sigma_{\Delta I})_m$ is a rectilinear func-

tion of ΔI_m , as in all such experiments.⁵ Figure 2 gives the band form of $\log I_m$ as a function of $\log \Delta I$. The kink in the plot is attributable to the duplex constitution of the retina, the lower segment representing rod functioning, the higher that of cones;⁷ the two are connected by a curved region, convex upward, suggesting rod-cone fluctuation of the sensory basis for the index-response in this region. (Individuals differ in the degree of this overlapping, and consequently in the curvature of the junction.)

The form of figure 2 requires that the variation of \bar{I} must exhibit a course totally different from that of $\Delta \bar{I}$, if the variation observed is in each case due primarily to the lawful fluctuating capacity of the organism. The relative variation of ΔI ($=\sigma_{\Delta I}/\Delta I_m$) is statistically constant (independent of I), and the vertical breadth of the band in figure 2 is therefore constant.

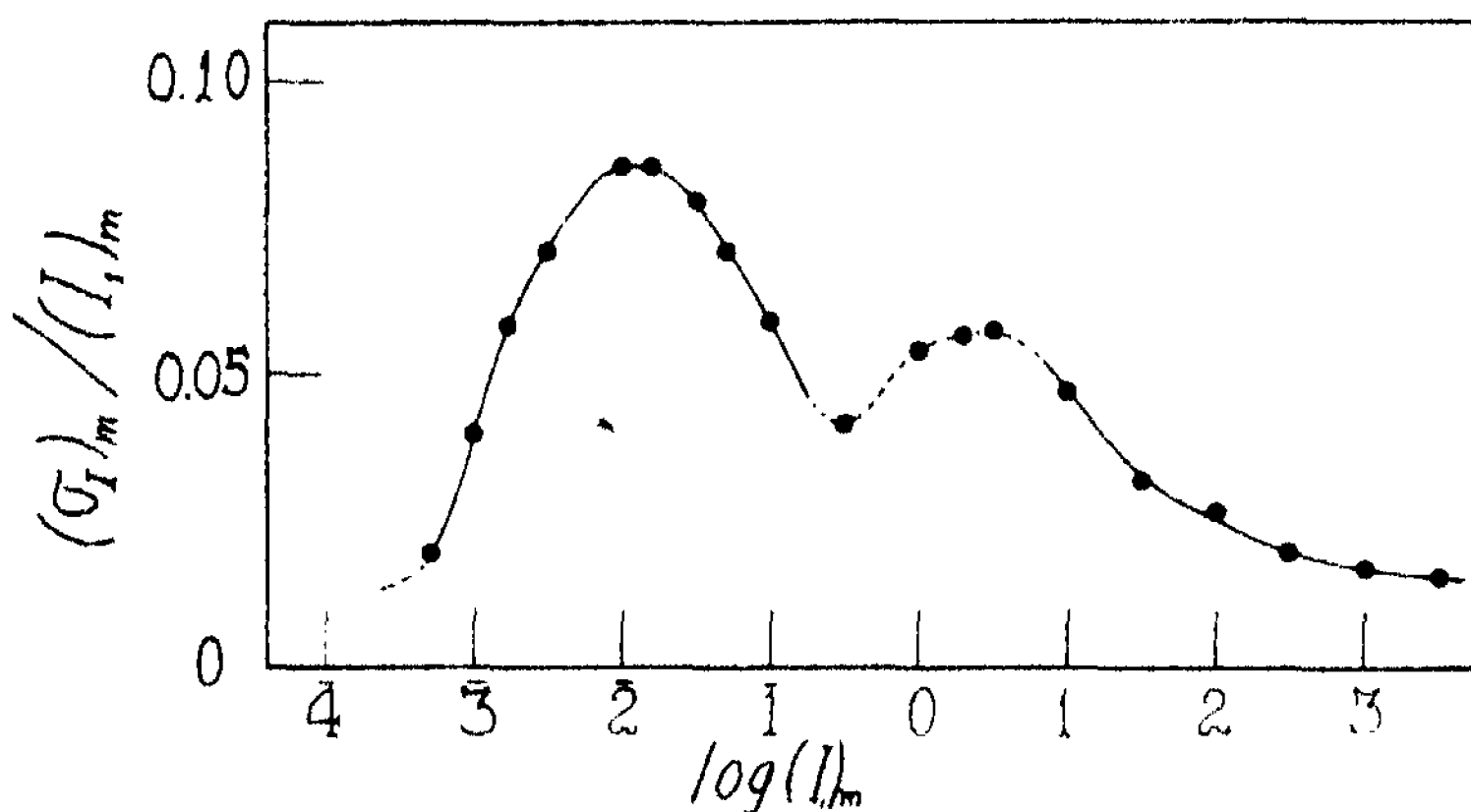


FIGURE 4

$(\sigma_I)_m/(I)_m$ as a function of $\log \bar{I}_1$, computed from the central line of figure 4. See text. To be contrasted with the properties of $\sigma_{\Delta I}$ (Fig. 1), where $(\sigma_{\Delta I})_m/(\Delta I)_m$ is found constant.

The horizontal breadth is not. We should find that as \bar{I} increases σ_I/I_1 rises to a maximum, declines, then rises to a minor maximum, since the band of figure 2 exhibits an inflection on either side of a major curvature. From equation (2), σ_I/I must be inversely proportional at each point to the slope of the band in figure 2.

Figure 3 gives the relation between σ_I and \bar{I}_m . As a function of $\log I_m$, $\log (\sigma_I)_m$ rises, with declining slope, to a point corresponding to the exclusive dominance of presumed "cone" effects, then rises with increasing steepness. The vertical breadth of the band in figure 3 (a measure of σ_{σ_I}) is necessarily proportional at each point to the slope of the band, but the proportionality factor is not the same for the two parts of the curve and hence for the two "universes" of effects—traditionally associated with rods and

cones respectively—from which the samples of measurement are drawn.

4. The test of equation (2) is made by computing σ_I/I from the central line of figure 3. Five series of measurements are plotted together in figure 3. The assemblage is not exactly homogeneous;⁴ because the whole function fluctuates from day to day, the central line does not vertically divide the σ_I span arithmetically in half.⁵ Figure 4 shows that σ_I/I does exhibit two maxima, as predicted. If it were possible experimentally to determine the lowest part of the $\Delta I - I$ band more completely by the method of observing \bar{I}_1 with ΔI fixed, which cannot be done because the visual threshold, the σ_I/I curve should rise again at the low I_1 end.

Summary.—When the minimal discriminable increment of intensity ΔI is measured as a function of \bar{I}_1 (i.e., $I_2 - I_1 = \Delta I$), the relative variation of ΔI is constant (independent of I_1). If, using the same manipulative procedure, \bar{I}_1 is determined as a function of fixed ΔI (by $\bar{I}_2 - \bar{I}_1 = \Delta I$), the relative variation of \bar{I}_1 is directly proportional to $d \log I / d \log \Delta I$, and is a complex function of \bar{I}_1 . This result is uniquely predicted by the principle that the observed variation is due primarily to the fluctuating reactive capacity of the organism.

¹ Crozier, W. J., *Déterminisme et variabilité*, Paris, Hermann, 56 pp. (1935) (and citations to earlier work); *Jour. Gen. Physiol.*, **19**, 503 (1935-36); *Proc. Nat. Acad. Sci.*, **22**, 412 (1936).

Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20**, 211 (1936-37); *Ibid.*, **21**, 17 (1937-38).

Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23**, 23 (1937).

Holway, A. H., and Crozier, W. J., *Ibid.*, **23**, 509 (1937).

² Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **21**, 17 (1937-38).

³ Crozier, W. J., and Holway, A. H. (in course of publication).

⁴ It is to be noted¹ also that the curve of \bar{I}_m as a function of ΔI cannot be identical with that for ΔI_m as a function of I . In the present experiment the difference between the areas of I_1 and I_2 complicate the comparison.

⁵ Crozier, W. J., *Jour. Gen. Physiol.*, **19**, 503 (1935-36).

Holway, A. H., *Jour. Opt. Soc. Amer.*, **27**, 120 (1937).

Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23**, 23 (1937).

⁶ A full description of the procedure will be given elsewhere.

⁷ Cf. Hecht, S., *Physiol. Rev.*, **17**, 239 (1937).

A DETERMINATION OF THE MAGNITUDE OF THE CELL
"SENSITIVE VOLUME" ASSOCIATED WITH THE
WHITE-EYE MUTATION IN X-RAYED
DROSOPHILA. III

BY C. P. HASKINS AND E. V. ENZMANN

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, AND THE HASKINS LABORATORY,
UNION COLLEGE

Communicated February 8, 1938

In previous communications¹ we have discussed the procedures used and the results obtained in a probability determination of the magnitude of the volume and the radius of the cell "sensitive volume" which appears to be associated with mutation at the white locus in *Drosophila melanogaster*. It has been indicated that the result may be tentatively considered as the minimum limiting value for the size of the gene.

Partly as a test of the method, and partly because of the wealth of interesting results which it has given, we have recently extended the scope of the investigation to include (1) an evaluation of the mutation rate-dosage relationship as a function of temperature of the irradiated material, using a single wave-length of x-rays, (2) an evaluation of the same relationship at

TABLE 1a
(MALES, 4°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)	CALCULATED TOTAL SENSITIVE VOLUME
0 min.	4646	0	0.00	±0.000	
1/2 min.	2888	3	0.10	±0.040	14.66 × 10 ⁻¹⁸ cc.
1 min.	1769	4	0.23	±0.077	16.87 × 10 ⁻¹⁸ cc.
2 min.	8964	53	0.56	±0.055	20.53 × 10 ⁻¹⁸ cc.
4 min.	2010	20	1.00	±0.467	18.32 × 10 ⁻¹⁸ cc.

TABLE 1b
(FEMALES, 4°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)
0 min.	4746	2	0.042	±0.020
1/2 min.	2972	7	0.27	±0.064
1 min.	1870	10	0.53	±0.113
2 min.	9436	70	0.75	±0.060
4 min.	2035	29	1.41	±0.176

^a It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases. This, because of the random distribution of modified patches in right and left eyes, is considered a justifiable procedure.

constant temperature with variable x-ray wave-length, (3) a determination of the same function at constant wave-length and room temperature when the locus "garnet" is substituted for "white" and (4) for the "white" locus at room temperature under neutron bombardment of constant velocity distribution. The first experiment is considered sufficiently complete to justify its report at this time. Work is in progress on the remaining ones.

per cent mosaics.

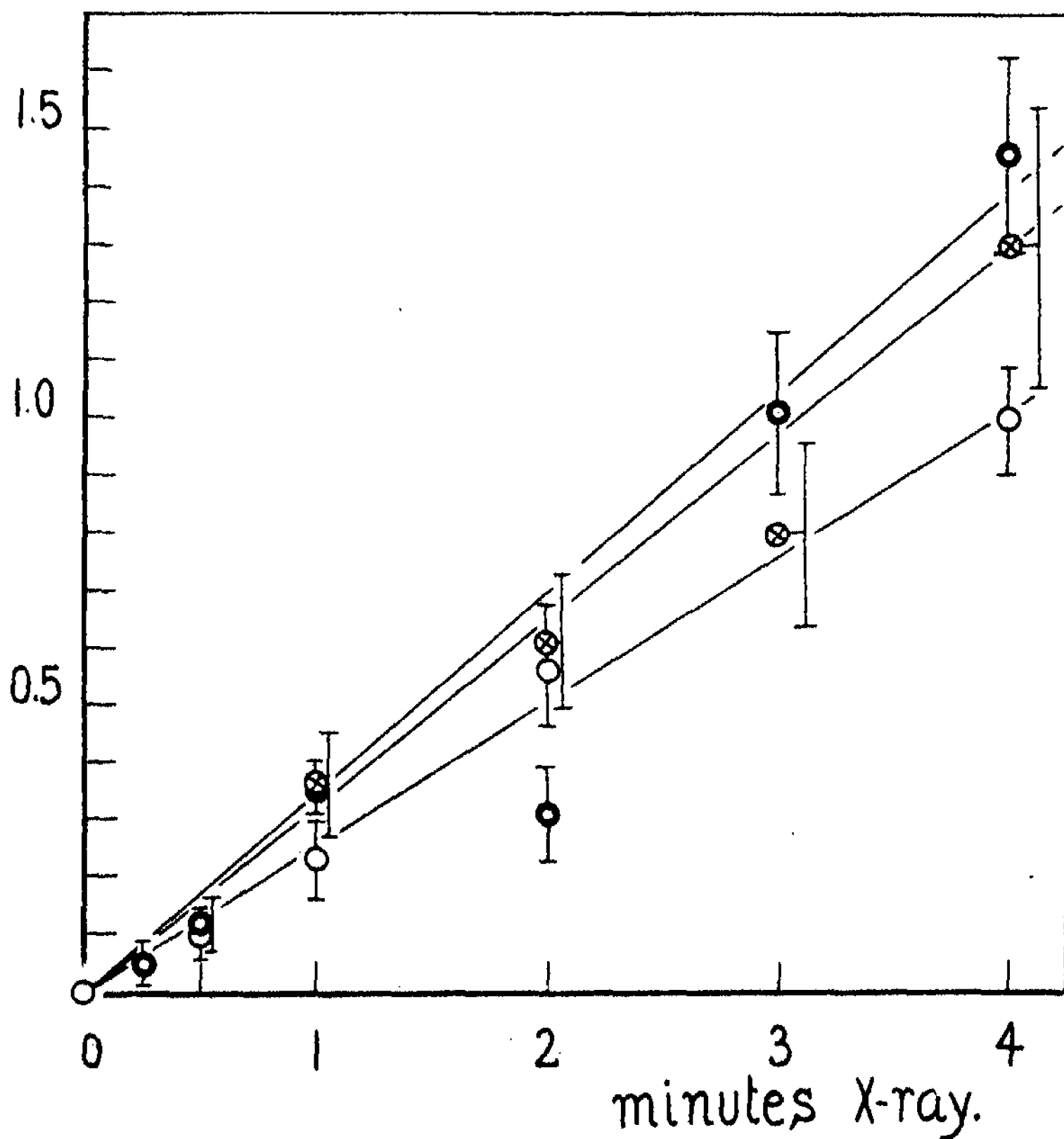


FIGURE 1

The experimental procedure reported in earlier papers was maintained unchanged, except that ova and young larvæ, eighteen to twenty-four hours old, were brought to temperature equilibrium with their surroundings at the values indicated before x-raying, held there during treatment and thereafter slowly returned to room temperature, at which the larvæ were reared. It was found possible to maintain eggs and larvæ at temperatures as low as

4°C. and as high as 54°C. Beyond these limits mortality resulting from temperature effects alone was unduly high.

The data of number of treated flies, number and percentage of affected eyes and percentage probable error involved, assembled separately for males and for females, are shown in tables I, II and III, for irradiation at temperatures of 4°C., 26°C. and 46°C., respectively. In figures 1 and 2 are shown the values for the percentage of affected eyes plotted arithmetically against the x-ray dosage. The justification of the arithmetic plot has earlier been considered.¹

TABLE IIa
(MALES, 26°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)	CALCULATED TOTAL SENSITIVE VOLUME
0 min.	4311	1	0.02	± 0.016	
1/4 min.	1781	1	0.05	± 0.036	14.66×10^{-18} cc.
1/2 min.	2583	3	0.11	± 0.044	16.13×10^{-18} cc.
1 min.	4610	16	0.35	± 0.054	25.66×10^{-18} cc.
2 min.	1960	6	0.31	± 0.085	11.33×10^{-18} cc.
3 min.	2188	22	1.01	± 0.144	24.68×10^{-18} cc.
4 min.	2237	32	1.46	± 0.171	26.76×10^{-18} cc.

TABLE IIb
(FEMALES, 26°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)
0 min.	4505	3	0.07	± 0.026
1/4 min.	1716	2	0.12	± 0.056
1/2 min.	2884	8	0.28	± 0.066
1 min.	4616	23	0.50	± 0.070
2 min.	2114	18	0.85	± 0.135
3 min.	1740	26	1.49	± 0.195
4 min.	2215	38	1.72	± 0.186

" It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases. This, because of the random distribution of modified patches in right and left eyes, is considered a justifiable procedure.

It will be seen that, as in the earlier experiments, a straight line may be considered the best fit for the points on all three of the curves representing data taken on males. This may be taken to indicate that the passage of a single electron through the locus considered is sufficient to accomplish the effect sought. As earlier set forth, this situation permits a calculation of the minimum limiting volumes of the sums of the loci for white eye in the x-chromosomes of all cells of the average optic anlage at the time of irradiation, at each of the three temperatures considered. These values, sepa-

rately calculated for each point on the three curves for males, are given in the final columns of tables Ia, IIa and IIIa.

The weighted means of these values, calculated in accordance with the

per cent mosaics.

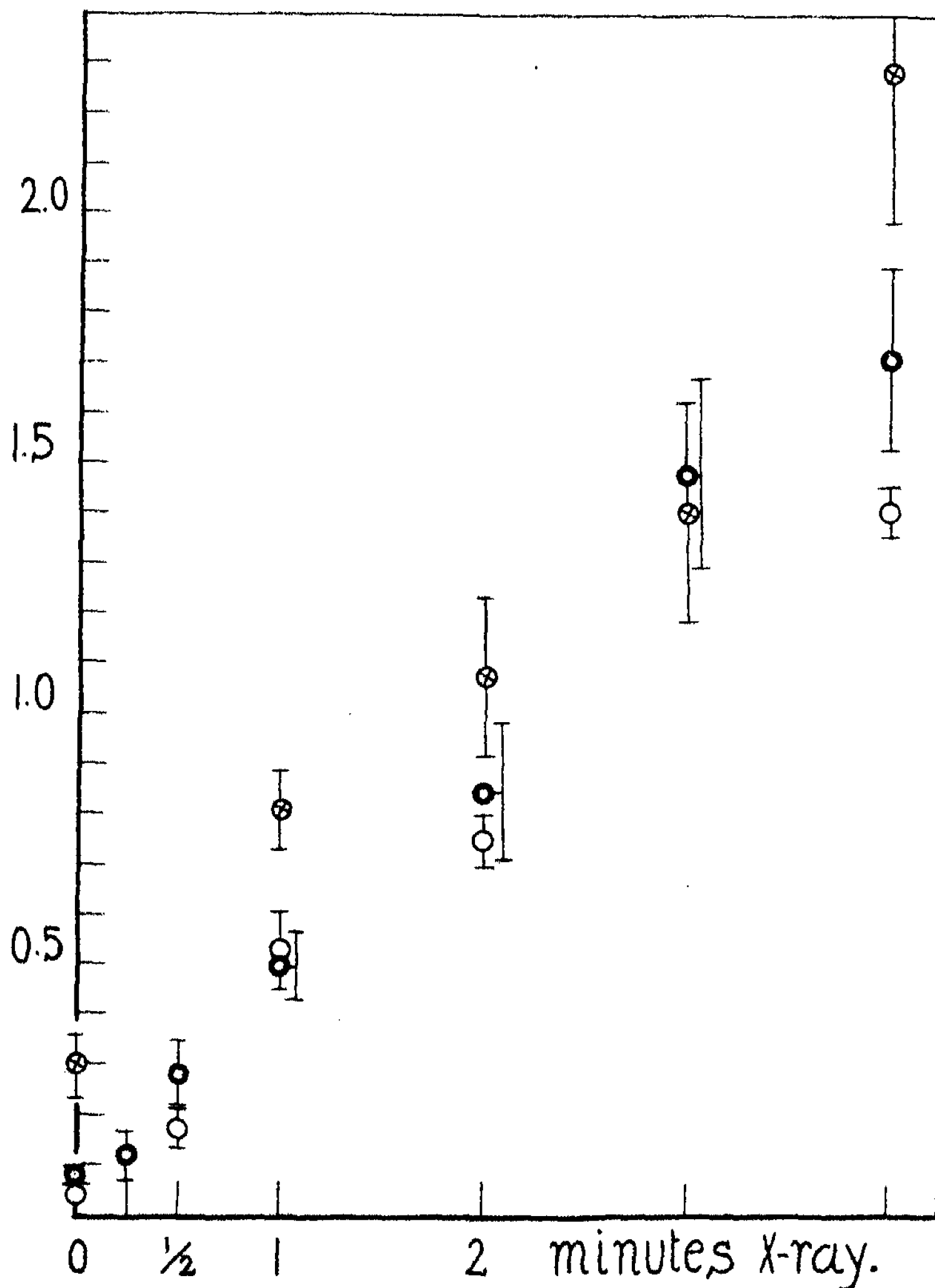


FIGURE 2

probable errors involved, are, respectively, 17.03×10^{-18} cc., 18.08×10^{-18} cc. and 23.36×10^{-18} cc. for 4°C ., 26°C . and 46°C . Our earlier work indicated that the figure 15.6 most nearly represented the average number of

cells in the optic anlagen of eosin larvæ of the stock used at the time of irradiation. Dividing by this number yields 1.09×10^{-18} cc., 1.16×10^{-18} cc. and 1.50×10^{-18} cc. as values for the minimum limiting volumes of single loci for white eye at the three temperatures considered. The corresponding radii, assuming arbitrarily a spherical form for the locus, are, respectively, 6.39×10^{-7} cm., 6.52×10^{-7} cm. and 7.10×10^{-7} cm., somewhat less than our earlier determination of 7.62×10^{-7} cm. as the minimum limiting radius at room temperature.

TABLE IIIa
(MALES, 46°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)	CALCULATED TOTAL SENSITIVE VOLUME
0 min.	2095	2	0.10	± 0.045	
1 min.	1796	7	0.36	± 0.095	26.39×10^{-18} cc.
2 min.	1812	11	0.61	± 0.123	22.36×10^{-18} cc.
3 min.	1371	11	0.80	± 0.162	19.55×10^{-18} cc.
4 min.	998	13	1.30	± 0.241	23.80×10^{-18} cc.
6 min.	542	11	2.03	± 0.408	24.80×10^{-18} cc.

TABLE IIIb
(FEMALES, 46°C.)

EXPOSURE	TOTAL NUMBER OF EYES ^a	NUMBER AFFECTED	PER CENT AFFECTED	PROBABLE ERROR (PERCENTAGE)
0 min.	1870	5	0.27	± 0.079
1 min.	1840	15	0.81	± 0.141
2 min.	1760	19	1.08	± 0.165
3 min.	1216	17	1.42	± 0.229
4 min.	1082	25	2.29	± 0.307
6 min.	451	11	2.44	± 0.480

^a It will be noticed that the total number of eyes given is sometimes odd. This situation results from the method used in counting. To facilitate rapidity, one eye only of each fly was counted in many cases.

The deviations between the calculated radii at 4° and 26°, and 26° and 46° are, respectively, 1.3 and 5.8 Ångströms. These differences considerably exceed the maximum probable errors for the entire series of measurements. It is interesting and possibly suggestive that the value for the higher temperature is in each case the larger, and collateral experiments have been begun to check the effect in monomolecular protein films. However, we should prefer to regard the deviations as without significance at present, since we have never demonstrated that the probability method is capable of giving results reliable to more than an order of magnitude. It may yet be worth while to point out that the new values show a closer numerical agreement with the values of the "radii" of molecules of various pro-

teins obtained by ultracentrifuge methods, referred to in an earlier paper, than did the figure given at that time.

The form of the curves for the data from females is of some interest. It is, of course, far from rectilinear, and it has been tentatively considered that this may be due, at least in part, to a confusion of whitened patches resulting from gross chromosome aberrations in the anlage cells with those arising as a result of true "point-locus" changes. The end results of both effects might well be morphologically indistinguishable in females, while in the males nearly all of the former are automatically eliminated through the death of the affected cell, since it becomes heterozygous for a gross x-chromosome aberration. That this may be the correct interpretation is indicated by the relatively close correspondence of lower dosage points for the three temperatures, and the marked deviation in the higher ranges, possibly caused by the known high temperature coefficient of aberrations. Such an effect is notably absent in the plots of data from males. It has been evident from the first that data from females cannot be used in sensitive volume calculations, except in the case of especially balanced stocks.

Further work is in progress concerning this and related questions.

¹ Haskins, C. P., *Proc. Nat. Acad. Sci.*, **21**, 561-566 (1935).

Haskins, C. P., and Enzmann, E. V., *Ibid.*, **22**, 397-400 (1936).

THE SPECIFICITY OF PYRIMIDINE FOR *PHYCOMYCES* *BLAKESLEEANUS*

BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

NEW YORK BOTANICAL GARDEN AND
DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated January 15, 1938

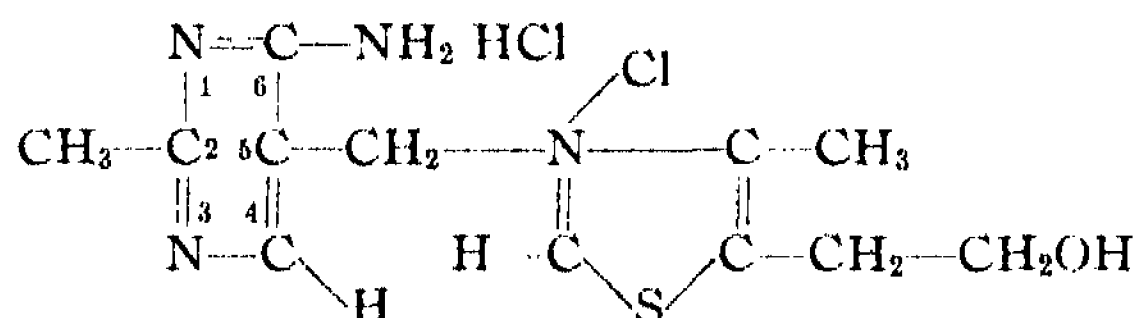
The authors¹ have reported that a mixture of the pyrimidine and thiazole intermediates used by Williams and Cline² in the synthesis of vitamin B₁ substitute for the vitamin in determining the growth of *Phycomyces Blakesleeanus* in a medium of mineral salts, dextrose and asparagine. Schopfer and Jung³ and Sinclair⁴ have found similar results. We have been interested in determining the specificity of the growth effect on *Phycomyces* of vitamin B₁ or its intermediates.

In an earlier paper¹ we reported negative results from the substitution for the vitamin thiazole of 15 sulfur compounds including some thiazoles. Negative results also were secured from the substitution of nucleic acid or its acid hydrolysate for the vitamin pyrimidine and in the substitution of pimelic acid or ethylene chlorhydrin for vitamin B₁.

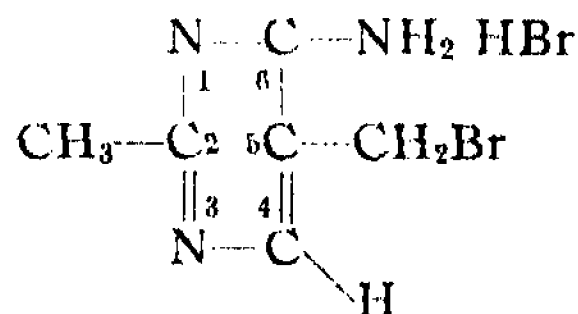
Since the previous report we have tested a number of additional compounds. Many of these compounds were secured through the generous co-operation of several individuals to whom we are deeply indebted.

The various pyrimidines tested were added to a medium ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g.; KH_2PO_4 , 15.0 g.; asparagine, 10.0 g.; dextrose, 100 g. and redistilled water, 1000 cc.) containing the vitamin thiazole. Twenty-five cc. of solution were used in 125-cc. Erlenmeyer flasks of Pyrex glass. Unless otherwise noted the solution and supplements were sterilized at 12 lbs. pressure for 20 minutes. After sterilization the solutions were inoculated with a suspension in redistilled water of the spores of *Phycomyces Blakesleeanus*, plus strain. For convenience in reference the various pyrimidines used or referred to are numbered.

The structure of vitamin B₁ hydrochloride is as follows:



On this basis 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide is



We have used this system in naming the various pyrimidines referred to in this report.

We have reported previously that the 2-methyl-5-bromo-methyl-6-aminopyrimidine hydrobromide No. (1) and the 2-methyl-5-ethoxymethyl-6-aminopyrimidine No. (2) appear to be equally effective. A mixture of pyrimidine No. (1) or No. (2) and the vitamin thiazole was found¹ to be as effective in determining the growth of *Phycomyces* as molecularly equivalent quantities of vitamin B₁. Schopfer and Jung³ and Sinclair⁴ found similar results with 2-methyl-5-amino-methyl-6-aminopyrimidine No. (3).

We secured negative results with 2-methyl-5-ethoxymethyl-6-oxypyrimidine No. (4) and with 2-methyl-6-aminopyrimidine No. (5) supplied by Merck and Co., and with 2:6-dichloro-5-chloromethyl-4-methylpyrimidine No. (6) and 2:6-dihydroxy-5-hydroxymethyl-4-methylpyrimidine No. (7) kindly furnished by A. R. Todd of the Lister Institute of Preventive Medicine, London. Each of these compounds was used in amounts of 1 unit⁵ and of 10 units with 10 units of the vitamin thiazole. A third compound

furnished by Todd, 2-methyl-5-thio-formamidomethyl-6-aminopyrimidine No. (8) was found partially to replace the vitamin pyrimidine. Sinclair found similar results but Schopfer⁶ reports it to be ineffective.

The difference between the results of Sinclair and ourselves and those of Schopfer is probably because the effectiveness of this compound is influenced by heating. In our experiments it was most effective when filtered sterile, less effective if heated for 20 minutes at 12 lbs. pressure and ineffective after two periods of 20 minutes autoclaving at 12 lbs. pressure. Used with 10 units of the vitamin thiazole, pyrimidine No. (8) when filtered sterile yielded for 1 unit 49 mgms. dry weight of mycelium, for 10 units 189 mgms. and for 100 units 300 mgms. In this experiment the growth with 10 units of the vitamin thiazole and 10 units of pyrimidine No. (2) was 518 mgms. The loss of effectiveness of pyrimidine No. (8) on heating is evidence that the growth secured with it was not because of contamination by other pyrimidines. We have found pyrimidines No. (1) and No. (2) in acid or neutral solution to withstand several autoclavings, and Sinclair found pyrimidine No. (3) to withstand 2 hrs. at 125°C. even in the presence of 0.1 *N* NaOH. We estimate from our results the effectiveness of pyrimidine No. (8) to be 50% or less than that of pyrimidines No. (1), No. (2) or No. (3).

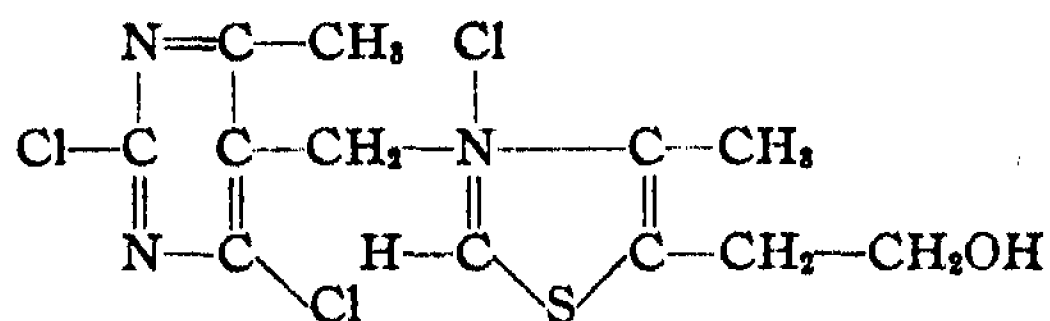
Ten units of each of the following pyrimidines were used with 10 units of the vitamin thiazole with negative results: 2:4 dimethyl-6-aminopyrimidine No. (9), 2:4 dichloro-5-chloromethyl-6 methyl pyrimidine No. (10), 2:4:6 trimethylpyrimidine dihydrate No. (11), 2-methylmercapto-4-methyl-6-hydroxypyrimidine No. (12), 2-phenyl-4-methylpyrimidine-6-carboxylic acid No. (13), 2-phenylpyrimidine 4:6 dicarboxylic acid No. (14), and 4-methyl-5 hydroxymethyl uracil No. (15). These seven compounds were furnished by Andrew Bowman of Oxford University.

A compound supplied by T. McLean of the Royal Technical College, Glasgow, 2-amino-5-methyl 1:3:4 thiadiazine hydrochloride No. (16), was used at 100 units with 10 units of the vitamin thiazole. Negative results were secured. The amount used was not entirely soluble. Negative results also were secured when compound No. (16) was used with 15 units of pyrimidine No. (1).

Fourteen pyrimidine compounds furnished by Treat B. Johnson of Yale University gave negative results. Ten units of each compound and 10 units of the vitamin thiazole were added to each flask. The compounds used were as follows: 2-thio orotic acid No. (17), 2-thio-4-methyl uracil No. (18), orotic acid No. (19), 5-iodo uracil No. (20), uracil-4-aldehyde No. (21), 2-thiouracil No. (22), 2-thio-4-methyl-uracil No. (23), uracil-5-carboxylic acid potassium salt No. (24), 1,4-dimethyl uracil No. (25), 2-thio-imino barbituric acid No. (26), 5-ethyl-4-methyluracil No. (27), 5-bromo uracil No. (28), 2-thio-4-hydroxymethyluracil No. (29), 4,5-dimethyluracil No. (30).

Schopfer⁶ has reported negative results with the following additional pyrimidines: 2-methyl-6-mercaptopyrimidine No. (31), 2:5-dimethyl-6-aminopyrimidine No. (32), 2:5-dimethyl-6-oxypyrimidine No. (33), 2-methyl-4-amino-6-hydroxypyrimidine No. (34) and 2:6-dioxypyrimidine (uracil) No. (35). Sinclair's results with 2-methyl-5-thioformamidomethyl-6-hydroxypyrimidine No. (36) and the vitamin thiazole were negative.

A compound No. (37) resembling vitamin B₁ was supplied by Bowman. As shown by the structural formula below the thiazole portion of the compound is identical with the vitamin thiazole but the pyrimidine portion is not.



Used in amounts of 1 unit and 10 units this compound was ineffective. It was also ineffective when filtered sterile and used in an amount of 10 units. However, the growth with 10 units of compound No. (37) and 10 units of pyrimidine No. (2) was comparable with that obtained with 10 units of vitamin B₁. These results were secured also when the compound was filtered sterile. We conclude that *Phycomyces* probably splits the compound into thiazole and pyrimidine. The vitamin thiazole thus obtained is then combined with pyrimidine No. (2) to form vitamin B₁. Schopfer obtained similar results with two compounds which contain the vitamin thiazole; namely, 3-benzyl-4-methyl-5 β -hydroxyethyl thiazole chloride and 3(4'[5']-methylimidazole)4-methyl-5 β hydroxyethythiazole hydrochloride.

The following conclusions seem justified on the relation between the structure of pyrimidines and their effectiveness when used with the vitamin thiazole on the growth of *Phycomyces*.

1. The amino group in the sixth position is important. This follows from the negative results with pyrimidine No. (4) where the amino group is replaced by oxygen.
2. The mono-substituted methyl group in the fifth position is important. This follows from the negative results with pyrimidine No. (5) where H replaces the methyl group and with pyrimidine No. (32) where the CH₃ group replaces the mono-substituted methyl group.
3. Various radicals may be attached to the methyl group in the fifth position as shown by the equivalent results with pyrimidines No. (1), No. (2) and No. (3) where the groups in the fifth position are CH₂Br, CH₂OC₂H₅ and CH₂NH₂, respectively.
4. The effectiveness is limited to some extent by the radical attached to the methyl group in the fifth position as shown by the results with pyrimi-

dine No. (8) when the CH_2NHCSH group reduced the effectiveness of the pyrimidine 50% or more as compared with that of pyrimidines No. (1), No. (2) or No. (3).

5. We have no evidence on the significance of the methyl group in the second position.

6. The pyrimidine portion of the vitamin B_1 molecule is highly specific for *Phycomyces*.

¹ William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499-502 (1937).

² R. R. Williams and J. K. Cline, *Jour. Am. Chem. Soc.*, **58**, 1504-1505 (1936).

³ William H. Schopfer and Albert Jung, *Compt. Rend. Acad. Sci. Paris*, **204**, 1500-1502 (1937).

⁴ H. M. Sinclair, *Nature*, **140**, 361 (1937).

⁵ 1 unit is 10^{-9} Mole of the compound in question.

⁶ W. H. Schopfer, *Bul. Soc. bot. suisse*, **47**, 460-464 (1937).

THE SPECIFICITY OF THIAZOLE FOR PHYCOMYCES BLAKESLEEANUS

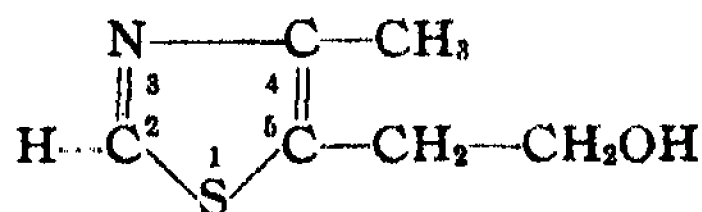
BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

NEW YORK BOTANICAL GARDEN AND
DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI

Communicated January 17, 1938

In a previous note¹ we have reported the results of a study of the specificity for *Phycomyces Blakesleeanus* of the pyrimidine portion of the vitamin B_1 molecule. In this paper the results with certain thiazoles are given.

The vitamin thiazole is 4-methyl-5 β hydroxyethylthiazole No. (1) and may be represented as follows:



A mixture of the vitamin thiazole and 2-methyl-5-ethoxymethyl-6-aminopyrimidine No. (1) or 2 methyl-5-bromomethyl-6-aminopyrimidine No. (2) is as effective as molecularly equivalent quantities of vitamin B_1 in determining growth of *Phycomyces*.²

Various thiazoles secured through the generous coöperation of various individuals were added to a medium ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g.; KH_2PO_4 , 15.0 g.; asparagine, 10.0 g.; dextrose, 100 g. and redistilled water, 1000 cc.) containing pyrimidine No. (1) or No. (2). Twenty-five cc. of solution were used in 125-cc. Erlenmeyer flasks of Pyrex glass. Unless otherwise noted the solutions were sterilized at 12 lbs. pressure for 20 minutes. After steri-

lization the solutions were inoculated with a suspension in redistilled water of the spores of *Phycomyces Blakesleanus*, plus strain. For convenience in reference the various thiazoles used or referred to are numbered.

Negative results were secured with the following thiazoles furnished by L. R. Cerecedo of Fordham University: 4-methylthiazole acetic acid No. (2), 4-methylthiazole-5-acetamide No. (3), ethyl-4-methylthiazole-5 acetate hydrobromide No. (4). Two amounts (1 unit³ and 10 units) of each of these were used with 10 units of pyrimidine No. (1).

Four thiazoles furnished by H. T. Clarke of Columbia University were used. They were 4-methyl-5 β -carbethoxy-ethylthiazole No. (5), 4-methyl-5 β -ethoxyethylthiazole picrate No. (6), 4-methyl-5 β -chloroethylthiazole picrate No. (7) and 2:4-dimethylthiazole pheniodide No. (8). Negative results were secured with thiazole No. (5) used in amounts of 1 unit and 10 units with 10 units of pyrimidine No. (1). Ten units of thiazole No. (8) with 10 units of pyrimidine No. (1) gave a yield of 3.3 mgms. dry matter. The results with thiazole No. (6) and No. (7) are shown in the following table. In each instance the thiazoles were used with 10 units of pyrimidine No. (1).

THIAZOLE NUMBER	AMOUNT UNITS	DRY WT. MYCELIUM MGMS.	
		FILTERED STERILE	HEATED
No.(6)	1	0	0
	10	46	26
	100	669	650
No.(7)	1	0	8
	10	59	60
No.(1)			
Vitamin	1.0	...	80
Thiazole	10	...	484

Ten units of thiazolidine-4-carboxylic acid No. (9) prepared as described by Ratner and Clarke⁴ were used with 10 units of pyrimidine No. (1) with negative results.

The following thiazoles were furnished by E. R. Buchman of the California Institute of Technology: 2:4-dimethyl-5 β hydroxyethyl thiazole No. (10), 4-methyl-5 β hydroxypropyl thiazole No. (11), and 4-methyl-5 γ hydroxypropyl thiazole No. (12). Two amounts of each, 1 unit and 10 units were used with 10 units of pyrimidine No. (1). Thiazole No. (10) appeared somewhat toxic. Thiazole No. (11) was ineffective and thiazole No. (12) gave 2% or less of the growth of a molecularly equivalent quantity of the vitamin thiazole. Thiazoles No. (11) and No. (12) were filtered sterile and 100 units of each used with 15 units of pyrimidine No. (1). The growth was less than 1% of that secured with the vitamin thiazole.

T. McLean of the Royal Technical College, Glasgow, supplied 2 keto-4 methyl-2:3 dihydrothiazole-2-benzylidene hydrozone No. (13). Negative

results were secured when 100 units of this compound were used with 10 units of pyrimidine No. (2).

Schopfer⁵ found the 4-methyl-thiazole No. (14), and the 4:5-dimethyl thiazole No. (15), were ineffective.

In addition we have tested nicotinic acid, neopeptone (Digestive Ferments Co.) and a sample of pantothenic acid kindly furnished by R. J. Williams. Nicotinic acid was found by Knight⁶ to be a growth factor for *Staphylococcus aureus*. In our experiments both nicotinic acid and pantothenic acid gave negative results as a substitute for vitamin B₁, thiazole or pyrimidine. On the other hand some growth was secured when neopeptone was substituted for vitamin B₁ or its intermediates. As shown in the following table, there seemed to be more thiazole than pyrimidine in the sample of neopeptone used.

NEOPEPTONE	PYRIMIDINE NO. 1 UNITS	VITAMIN THIAZOLE UNITS	DRY WT. MYCELIIUM MGMS.
0.1 g. per l.	0	0	11.3
0.1 g. per l.	10	0	30.5
0	10	10	350.0

The following conclusions seem justified on the relation between the structure of thiazole and its effectiveness when used with a suitable pyrimidine on the growth of *Phycomyces*.

1. The H in the second position is important. This follows from the negative results with thiazole No. (10) in which the H is replaced by CH₃.

2. The β hydroxyethyl group in the fifth position is important. This follows from the negative results with thiazole No. (2), No. (3), No. (4), No. (5), No. (11), No. (12), No. (14) and No. (15) in which the β hydroxyethyl group is replaced by the acetate, acetamide, ethylacetate, β carbethoxyethyl, β hydroxypropyl, γ hydroxypropyl, hydrogen or methyl radicals respectively.

3. The hydroxyl in the hydroxyethyl group is important. This follows from the materially decreased growth with thiazoles No. (6) and No. (7) in which the hydroxyl is replaced by the ethoxy radical or by chlorine.

4. We have no evidence as yet on the significance of the CH₃ group in the fourth position.

5. The thiazole portion of the vitamin B₁ molecule is highly specific for *Phycomyces*.

¹ William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **24**, 141-145 (1938).

² William J. Robbins and Frederick Kavanagh, *Ibid.*, **23**, 499-502 (1937).

³ 1 unit is 10⁻⁹ Mole of the compound in question.

⁴ Sarah Ratner and H. T. Clarke, *Jour. Am. Chem. Soc.*, **59**, 200-206 (1937).

⁵ W. H. Schopfer, *Bul. Soc. bot. suisse*, **47**, 460-464 (1937).

⁶ B. C. J. G. Knight, *Biochem. Jour.*, **31**, 966-973 (1937).

NOTE ON THE PROBLEM OF THE EXPANDING UNIVERSE

BY HARLOW SHAPLEY

HARVARD COLLEGE OBSERVATORY

Communicated February 14, 1938

1. Question as to the proper interpretation of the red-shift has been raised through the publication of nebular counts by Hubble.¹ If we assume that the galaxies are in the main uniformly distributed in space, we have the relation $\log N_m = 0.6 (m - m_1)$ between the number of objects per square degree and the apparent magnitude limit m (corrected for red-shift). The constant m_1 is the space-density parameter, that is, the magnitude to which a survey must penetrate to record on the average one object per square degree. Its evaluation is necessary in the calculation of the effect of red-shift on magnitude; and the size of this effect determines whether or not the red-shift means an expanding universe, or demands some other as yet unspecified interpretation.

The value of m_1 cannot be very securely determined by means of the thirteen hundred galaxies brighter than the thirteenth magnitude, because not only is the number involved relatively small, but also the distribution is dominated in some parts of the sky by clusters of galaxies, and in others by irregular space absorption. Nor can m_1 be precisely determined as yet by the use of objects fainter than the nineteenth magnitude, where difficulties arise through the increasing size and significance of the red-shift correction and increasing uncertainty in the nebular magnitude scale.

Between the thirteenth and nineteenth magnitudes the red-shift correction is either negligible or not seriously uncertain, and in that interval the stellar photographic magnitude scale, on which the nebular magnitudes are based, is most satisfactorily determined. It is here, therefore, that m_1 can best be derived from nebular counts and from photometric measures.

A recently completed survey of the galaxies to the eighteenth magnitude in the south galactic polar cap provides extensive material for the determination of m_1 . Also this new survey gives a picture of the distribution of galaxies over an extended region of the sky that appears to be quite clear of irregularities in space absorption and not obviously affected by distinct clusters of galaxies. The details of the investigation will be presented in Circulars and Annals of the Harvard Observatory; the present account will deal only with the determination of m_1 and related quantities.

2. Eighty plates of three hours exposure have been made in the polar area bounded by galactic latitude -55° ; all but nine are in the zone bounded by $\beta = -60^\circ$. The plates are of fairly uniform quality, the mean limiting stellar magnitude being $m_s = 18.36 \pm 0.04$ (m.e.). Each plate, fourteen inches by seventeen inches, covers approximately thirty-five

square degrees. In the central part of the plate (three degrees on a side) there is no appreciable magnitude loss that depends on distance from the optical axis, for either stars or galaxies. In the following discussion of the nebular counts and the nebular distribution, the argument is based chiefly on these central nine square degrees, in which we find on the average about fifty galaxies per square degree.

In the surrounding sixteen square degrees we find, in the mean, only thirty-one galaxies per square degree, indicating that the limiting magnitude for galaxies at this distance from the center of the plate is between three- and four-tenths of a magnitude brighter than at the plate center. These outer squares can be used effectively in studying the unevenness of the nebular distribution from plate to plate over the polar zone, as well as in the general cataloguing of external galaxies.

3. The plates for the present survey were made with the 24-inch Bruce doublet at Bloemfontein. The magnitudes have been determined on each plate separately by the star-count method. The nebular counts are summarized in table 1.² The last column gives for each plate the number of galaxies per square degree averaged over the central nine square degrees after the plate is reduced to a common magnitude limit. This reduction has not appreciably affected the total numbers involved, and has decreased the differences in magnitude limit from plate to plate that arise from variations in sky conditions and emulsion speeds. Since the magnitudes are determined independently on each plate, no adjustment is needed for differences in zenith distance.

4. From the 36,274 objects actually observed within the 9-square areas of the eighty plates, we deduce, when each plate is treated as a uniform unit, the mean value and its mean error

$$\bar{N}_m = 51.51 \pm 3.11$$

The limiting nebular magnitude m is found (from a study of the magnitude-frequency plots for the galaxies on a hundred long-exposure Bruce plates, mostly in the zone³ south of declination -60°) to be 18.20 with an estimated mean error of less than $0^m.05$. The red-shift correction is taken as $0^m.16$ (from a discussion by Hubble), and the corresponding space-density parameter is computed to be

$$m_1 = 15.19,$$

with an estimated mean error of $\pm 0^m.06$. This value of m_1 is in fair agreement with the values of 15.3 and 15.09 derived by Mayall⁴ and Hubble,⁵ respectively. All three determinations, of course, are computed on the hypothesis of uniform space density.

The standard deviation from the mean \bar{N}_m for a single plate in table 1 is 27.67, indicating the considerable irregularity in nebular distribution

throughout the south galactic cap, even when areas as large as nine square degrees are involved. The distribution of \bar{N}_m is of course not symmetrical, the quartile, median and three-quartile values being 30.2, 44.4 and 64.0, respectively. The mean of the three largest values of \bar{N}_m is ten times the mean of the smallest three, corresponding, on the uniform space-density hypothesis, to a difference in m_1 of 1.67 magnitudes—a difference that is quite out of the question as an error in determining magnitude limits, and indicates rather the degree of non-uniformity that may be found when neighboring areas in high latitudes, each of twenty-seven square degrees, are compared.

5. Because of the clustering tendency of galaxies, it is best not to examine the spread of values of the parameter m_1 only on the basis of richest and poorest plates, but rather to deal with large areas. If we divide the polar cap into eastern and western halves, containing respectively the second and third longitude quadrants and the first and fourth longitude quadrants, we derive from the data of table 1 the results of table 2. Again each plate is treated as a uniform unit and the data are used from the central nine square degrees only.

TABLE 2

	EAST	WEST
Number of plates	38	42
Area in square degrees	342	378
Total number of identified galaxies	19,617	16,657
Mean \bar{N}_m (reduced to $18^m.2$)	60.82	43.14
Standard deviation	33.47	25.90
Mean error of mean	5.50	4.04
Median \bar{N}_m	51.75	37.15
Quartile values of \bar{N}_m	39.4, 69.6	26.7, 51.8
Mean of 3 largest \bar{N}_m	154.5	104.9
Mean of 3 smallest \bar{N}_m	20.2	15.7
Space-density parameter m_1	15.07	15.32

A similar difference in the values of m_1 would be obtained if we divided the polar area into northern and southern halves; but it is better, in order to avoid a possible systematic error, to divide as above, because the stellar magnitude limits have been deduced by slightly different methods for regions north and south of declination -23° . There appear to be no factors of an observational, seasonal or computational nature that can account for an appreciable amount of the differences in the nebular count for the two half-zones. We conclude that the observed difference in average population per square degree, namely $60.82 - 43.14$, is an indication of a metagalactic structural condition. The eastern half of the absorption-free polar cap is 1.41 times as rich as the western half.

6. It should be noted that the areas covered, 342 and 378 square degrees, as well as the numbers of galaxies observed, 19,617 and 16,657, are

considerably larger than the areas and numbers in any of the five surveys made with the reflectors at the Mount Wilson and Lick Observatories that have been used by Hubble in the analyses of the distribution of galaxies in space. From Hubble's table 1 in the paper referred to above¹ we derive the following values:

To magnitude limit	18.47	19.0	19.4	20.0	21.03
Total of nebulae actually identified	2,299	9,940	8,774	11,856	8,200
Total effective area* in square degrees	30	(69)	40	24.3	5.6

* The "effective" area for a survey, in square degrees, is the average number of nebulae actually identified per plate, divided by the average number per square degree (reduced to standard conditions) multiplied by the number of plates.

Except perhaps for the two brightest limits, the intercomparison of areas involved is not significant. It is the volumes of space that should be inter-compared, or, better yet, the total number of galaxies observed.

7. When the four quadrants of the south galactic cap are treated separately, we find from the Harvard material:

Quadrant	1	2	3	4
Number of plates	18	18	21	23
Number of galaxies	5,002	8,708	10,909	11,655
m_1	15.54	15.15	15.00	15.19

8. The material discussed above from the Harvard survey can be more than doubled by using the 39,680 other galaxies that are observed on the same plates in the sixteen square degrees outside the central nine square degrees. When the plates are reduced as before to a common stellar magnitude limit, the average number of galaxies in this outer area per square degree (each plate again treated as a uniform unit in computing the uncertainty) is

$$\bar{N}_m = 33.03 \pm 2.18 \text{ (m.e.)}.$$

The standard deviation for one plate is 19.36. The mean nebular magnitude limit for the sixteen square degrees is approximately 17.87 instead of the 18.20 applicable to the central nine square degrees. This value is arrived at simply by the comparison of the nebular counts:

$$\begin{aligned} m_9 - m_{16} &= 1.67 \log (\bar{N}_9 / \bar{N}_{16}) \\ m_{16} &= 18.20 - 1.67 \log (51.5 / 33.0) \end{aligned}$$

Since uniform space density is assumed for this calculation of m_{16} , the values of \bar{N}_m and m_{16} have no independence in the computation of the parameter m_1 ; but the data from these outer sixteen square degrees for the eastern and western halves of the polar cap can be used with high weight for an independent examination of the values of m_1 obtainable in two large adjoining areas. Table 3 contains the material for the outer

areas, comparable in detail with table 2. The eastern half is 1.50 times as rich as the western half, and the corresponding difference in the space-density parameter is 0^m.30.

TABLE 3

	EAST	WEST
Number of plates	38	42
Area in square degrees	608	672
Total number of identified galaxies	22,810	16,870
Mean \bar{N}_m (reduced to 17 ^m .87)	40.07	26.67
Standard deviation	22.53	13.01
Mean error of mean	3.70	2.03
Median \bar{N}_m	37.9	24.0
Quartile values of \bar{N}_m	25.0, 43.1	16.9, 30.7
Mean of 3 largest \bar{N}_m	100.9	56.7
Mean of 3 smallest \bar{N}_m	11.9	9.1
Space-density parameter m_1	15.06	15.35

9. On the hypothesis of strictly uniform space density, m_1 is a constant and the observed differences in \bar{N}_m would need to be attributed to corresponding differences in the magnitude limit m . But discrepancies of this size and systematic character in the magnitude limit for adjacent parts of the polar cap are not credible. The fault undoubtedly lies in the assumption of uniform space density. The observed inequality can be found also in Hubble's survey to magnitude 18.47, since twenty of his reflector plates are in the first, twenty-two in the second quadrant, with latitudes between -55° and -90° . He records 352 objects in the first, 562 in the second, and we find that the ratio of East to West is 1.45 for equal areas, in agreement with the results from Harvard plates (Sections 6 and 8 above). It may be noted that still larger inequalities are known than those in the south galactic cap. We hope to make a report soon on one or two of the great metagalactic clouds.

10. Finally we consider the bearing of the new data on the question of the effect of the red-shift on magnitude, given by the relation $\Delta m = B \frac{d\lambda}{\lambda}$. The factor B should be approximately 4.0 if the red-shift is due to recession, and 3.0 if recession is not involved. Both Eddington⁶ and Hubble⁷ have pointed out that B could be changed from Hubble's derived value of 2.94 to the value 4.0 with a relatively small change in the interval δm between the assigned magnitude limits of the various surveys. Thus Hubble notes that "an error in δm of the order of 0^m.12, evenly divided between the two extreme limits, is required to furnish $B = 4.0$."

For his calculations at the brighter limit, 18.47, Hubble determines $\log N_m$ from the counts of 2300 objects. When the new survey at a corresponding bright limit is used for deriving m_1 and B , we find the 42,400 galaxies of the eastern half of the south galactic cap give a value of m_1 that is nearly

three-tenths of a magnitude different from the value derived for the 33,500 galaxies of the western half. The same difference carries over to m and δm . It appears, therefore, that the coefficient B cannot be accurately determined from existing data, and that we have no need as yet, from nebular

TABLE 1

CENSUS OF 75,900 GALAXIES IN THE SOUTH GALACTIC POLAR CAP

GA- LACTIC LONGI- TUDE	GA- LACTIC LATI- TUDE	N_{tot}	N_0	N_{16}	\bar{N}_0	GA- LACTIC LONGI- TUDE	GA- LACTIC LATI- TUDE	N_{tot}	N_0	N_{16}	\bar{N}_0
1.5	-67.3	971	363	456	53.2	207.0	-57.3	581	193	288	85.3
2.7	-60.3	486	166	231	36.9	207.1	-78.2	382	157	144	15.2
7.8	-76.2	250	142	99	27.4	213.4	-75.5	1054	431	480	47.9
14.2	-72.8	262	104	99	46.0	214.2	-62.6	1538	665	668	64.3
14.4	-80.6	666	235	324	30.0	222.4	-69.9	310	125	131	27.8
23.3	-63.4	802	300	381	25.3	226.3	-57.4	658	260	290	66.2
24.2	-77.8	255	101	104	16.9	226.5	-75.7	1046	448	485	49.8
39.4	-64.0	385	212	145	17.9	227.7	-63.8	1917	930	800	51.7
39.6	-71.1	1344	537	641	45.3	235.0	-55.6	1226	503	523	147.0
51.6	-56.2	378	131	190	25.3	238.8	-75.2	1463	650	628	72.2
55.1	-63.1	530	214	253	23.8	239.0	-72.1	798	420	331	46.7
60.5	-69.7	645	278	288	23.5	239.7	-69.2	1830	767	808	64.7
64.4	-83.9	739	284	316	20.8	240.3	-81.8	1772	668	840	18.4
68.0	-76.4	1877	793	847	44.0	252.4	-60.7	2208	784	1097	151.6
68.7	-60.6	684	274	335	15.2	253.7	-66.5	1818	658	878	63.6
76.9	-66.8	855	270	427	39.6	258.6	-72.5	1632	561	876	31.2
88.5	-72.3	687	293	279	37.4	264.3	-62.5	1197	514	533	99.5
89.5	-62.3	896	308	356	45.2	270.3	-67.7	1968	832	896	92.4
90.0	-87.8	2631	863	1489	63.3	270.9	-77.7	1931	807	830	68.1
102.8	-66.6	583	216	294	55.0	274.1	-55.0	896	328	432	31.7
110.1	-76.6	4519	1730	2127	48.1	274.8	-87.7	1730	807	712	34.1
110.5	-60.4	1318	466	640	51.8	276.3	-62.4	1954	812	838	90.2
111.9	-63.1	949	415	419	69.6	282.1	-72.1	864	335	340	42.8
120.3	-70.2	962	317	479	40.5	286.9	-66.4	2575	1102	1106	80.8
124.7	-63.0	905	399	416	38.6	287.7	-60.6	1026	451	372	66.1
127.3	-56.5	811	289	412	32.1	299.3	-63.0	4025	2050	589	132.1
139.6	-71.4	1040	409	464	60.0	300.8	-68.9	419	169	208	16.3
140.5	-64.2	794	264	413	38.7	302.1	-75.0	1457	617	644	39.8
141.5	-82.1	678	227	339	57.8	315.8	-60.9	1087	465	484	89.9
147.2	-85.0	968	355	457	30.0	316.7	-78.7	1043	422	474	54.0
149.9	-55.5	717	280	350	27.1	318.2	-69.2	981	328	450	36.4
156.1	-63.7	2010	853	897	164.9	325.5	-62.3	785	316	337	26.7
156.8	-78.1	457	228	184	50.7	326.6	-75.1	603	226	268	43.7
160.8	-71.1	664	230	345	51.2	331.6	-71.3	890	359	433	30.3
170.6	-62.1	1927	1012	689	56.2	334.8	-67.5	696	245	333	35.9
178.5	-67.8	479	155	262	39.4	337.6	-55.1	946	309	483	51.8
192.0	-72.6	1215	527	564	77.3	342.1	-61.7	203	61	114	15.5
192.1	-63.8	1725	861	730	83.2	342.5	-80.7	547	205	242	30.1
197.2	-81.0	271	73	128	42.6	347.7	-72.8	666	264	279	38.7
202.6	-59.5	1824	714	912	91.2	352.4	-60.6	486	145	235	28.0

counts, to question the interpretation which attributes red-shift to recession.

The result can best be shown numerically, through least-squares solutions for m_1 and B , which are related (when we adopt the velocity-distance factor derived at Mount Wilson) by the formula⁸

$$m_1 = m_0 - 1.667 \log \bar{N}_m - 10^{0.2(m_0 - \Delta m_0) + \log B - 4.707}$$

where m_0 and Δm_0 are the observed magnitude limit and the red-shift effect. We use all of the survey material presented by Hubble (Section 6 above) except that we substitute the Harvard counts to 18^m.2 (nine inner squares only) for his data at the brighter limit, and obtain the following results for the eastern and western parts of the galactic cap:

	EAST	WEST
$\log \bar{N}_m$	1.784	1.635
m_1	15.01	15.26
B	3.48	1.81

If we should assume that the limit of completeness of the Harvard survey is 18.1 instead of 18.2, we find:

	EAST	WEST
m_1	14.90	15.19
B	4.2	2.2

¹ See, for example, these PROCEEDINGS, 22, 621-627 (1936).

² Detailed tabulations are given in Harvard Circular 423 (*in press*).

³ *Harv. Ann.*, 105, No. 8, p. 137 (1937).

⁴ *Lick Obs. Bull.*, 16, 177 (1934).

⁵ *Mount Wilson Contr.* No. 557, p. 15 (1936). Hubble's C is $-0.6 m_1$.

⁶ *Mon. Not. R. A. S.*, 97, 156 (1937).

⁷ *Ibid.*, 97, 506 (1937).

⁸ The form of the exponential term is suggested by Hubble.

DEPENDENT PROBABILITIES AND SPACES (L)

BY GARRETT BIRKHOFF

HARVARD UNIVERSITY

Communicated February 1, 1938

1. *Introduction.*—The purpose of the present paper is to express the theory of dependent probabilities in new terms.

These terms are: (a) the general theory of linear operators on Banach space, and (b) the relation $f < g$. The abstract theory of partially ordered Banach spaces has already been formulated by Kantorovitch;¹ it involves such notions as: upper bound, lower bound, least upper bound or sup,

greatest lower bound or inf, lim sup, lim inf, positive part, negative part, absolute value and disjointness.

Firstly, the fundamental definitions are stated in a form which includes all known cases; this has never been done before. Secondly, Markoff's fundamental theorem on "probabilities in chain" is proved in a form including all known cases. And lastly, a new theorem, which specializes in the *deterministic* case to von Neumann's well-known Mean Ergodic Theorem,² is proved with added generality in the *stochastic case*.

The present paper merely sketches the proofs, which will be given in full elsewhere.

2. *Postulates*.—The model with which we shall work is described in the following:

DEFINITION 1: By a space (L) , we mean any space Σ which satisfies the following six postulates.

P0: Σ is a linear space with real scalars, and a relation $f > 0$ (to be read, f is positive), is defined on Σ .

P1: If $f > 0$ and $g > 0$, then $f + g > 0$.

P2: If $f > 0$ and λ is a scalar, then $\lambda > 0$ implies $\lambda f > 0$ and conversely.

P3: Relative to the definition, $f > g$ (read, f is greater than g) means $f - g > 0$, Σ is a *lattice*.³

P4: A "norm" $\eta(f)$ is defined on Σ , relative to which Σ is a Banach space.⁴

P5: Norm is additive on positive elements; $f > 0$ and $g > 0$ imply $\eta(f + g) = \eta(f) + \eta(g)$.

Example: Let B denote the Boolean algebra of all subsets X of a class I_n of n points, or of Borel subsets of an interval I (or of any region!) modulo sets of measure zero. Then the additive, continuous functions defined on B satisfy (P0)–(P5), if by $f > 0$ we mean $f(X) \geq 0$ for all X and $f(I) > 0$, and by $\eta(f)$ we mean $f(I)$ when $f > 0$ and $\sup |f(X)| + |f(X')|$ in general. Thus the space (L) and its finite-dimensional analogues are "spaces (L) ."

Consequences of (P0)–(P2): Define $f > g$ to mean $f - g > 0$. Then (1) Σ is a partially ordered set in the sense of Hausdorff, (2) translations $x \rightarrow x + a$ preserve order, (3) homothetic expansions $x \rightarrow \lambda x$ preserve or invert order according as $\lambda > 0$ or $\lambda < 0$. We note that by (1)–(2), (P3) follows from (P0)–(P2) and the assumption that any element f has a "positive part" $f^+ = f \vee 0$, such that $x \geq f^+$ implies $x \geq 0$ and $x \geq f$ and conversely.

Consequences of (P0)–(P3): If we set $f^+ = f \vee 0$, $f^- = f \wedge 0$, and $|f| = f^+ - f^-$, then (4) the "Jordan decomposition" $f = f^+ + f^-$ holds, whence $f + g = (f \vee g) + (f \wedge g)$ by (2), (5) $f^+ \wedge (-f^-) = 0$ —in words, f^+ and $-f^-$ are *disjoint*, (6) the dual distributive laws $f \vee (g \wedge h) = (f \vee g) \wedge (f \vee h)$ and $f \wedge (g \vee h) = (f \wedge g) \vee (f \wedge h)$ are valid, (7) the triangle law on absolute values holds: $|f - g| + |g - h| \geq |f - h|$, (8) the functions $f \vee g$ and $f \wedge g$ are *monotone* in both variables, and *uniformly continuous* in that $|(f \vee g) - (f^* \vee g)| \leq |f - f^*|$ and $|(f \wedge g) - (f^* \wedge g)| \leq |f - f^*|$.

Consequences of (P0)–(P5): (9) The functions $f \cup g$ and $f \cap g$ are metrically continuous, in virtue of (8), (10) the functional $\lambda(f) = \eta(f^+) - \eta(f^-)$ is linear, and $\eta(f) = \lambda(|f|)$, (11) every set of elements of Σ having an upper bound has a least upper bound (and dually).

These results can be found in Kantorovitch, op. cit.

3. *Normal Subspaces and Decompositions.*—In proving our Mean Ergodic Theorem (Theorem 3), but not elsewhere, we shall want two further definitions, which seem to be new.

DEFINITION 2: A subspace of a linear space satisfying (P0)–(P3) is called “normal” if and only if it contains (a) with any f , also $|f|$, and (b) with any positive f , all “parts” x of f (i.e., all positive x with $x < f$).

DEFINITION 3: By a “direct decomposition” of Σ , is meant a choice of complementary normal subspaces—that is, of subspaces S and T such that $S \cap T = 0$, $S + T = \Sigma$.

Remarks: (1) The normal subspaces of Σ correspond to its homomorphisms in just the same way that the normal subgroups of a group correspond to its homomorphisms, and (2) the decompositions of Σ correspond one-one to its representations as a direct union.

4. *Connections with Dependent Probabilities.*—Spaces (L) are connected with the theory of dependent probabilities by three fundamental definitions.

DEFINITION 4: By a “distribution” is meant a positive element of Σ with norm one.

DEFINITION 5: By a “transition operator” on Σ is meant an additive operator which carries distributions into distributions.

DEFINITION 6: A transition operator T describing the dependence of the state of a system at time t' on its state at a previous instant t , is called “independent” of an operator U relating the instants t'' and t' [$t'' > t'$], if and only if the instants t'' and t are related by the transition operator TU : $f \rightarrow (fT)U$.

As authorities for these definitions, we can cite the usual formulations of Bayes' Theorem, of the theory of Markoff chains, of Kolmogoroff's more general theory of “stochastic processes.” Also, Fourier's theory of heat flow is expressed by transition operators: the invariance of $\lambda(f)$ is the gist of the first, and that of the set of $p \geq 0$ of the second, law of thermodynamics. Finally, the flows of phase-space envisaged by Poincaré in his version of classical mechanics, induce automorphisms on the space (L)—and hence are transition operators in our sense, as well as “unitary operators on Hilbert space.”

Conclusions: (1) The set Δ of all distribution functions is a closed convex subset of Σ , of diameter $\sup |(p - q)| \leq 2$, (2) the distance $\eta(|p - q|)$ is the “stochastic distance” recently defined by Mazurkiewicz—it is not equivalent to the traditional notion of “convergence in probability,” (3)

$|fT| \leq |f|$, whence $\eta(fT) \leq \eta(f)$ (T is of "modulus" unity, and a "contraction," and so uniformly continuous!).

5. *Hypothesis of Markoff*.—Now let Σ be any space (L), and T a fixed transition operator on Σ .

DEFINITION 7: An f in Σ is called a "fixpoint" if and only if $fT = f$. A distribution which is a fixpoint is called "stable."

Results: (1) The fixpoints are a closed linear subspace of Σ . Hence the stable distributions are a closed convex subset of Δ , and their number is either zero, or one (the "metrically transitive" case⁵), or infinity. All three cases are possible, but the second is the most interesting.

Hypothesis of Markoff (weakened): For some n , $d = \inf_{p \in \Delta} pT^n > 0$.

THEOREM 1: If T satisfies Markoff's hypothesis, then there is a unique stable distribution p_0 . Moreover the pT^k tend to p_0 uniformly, with the rapidity that the terms of a convergent geometrical progression tend to zero.

Proof: First, $\eta(pT^n - qT^n) \leq (1 - |d|)\eta(p - q)$ for any $p, q \in \Delta$. The conclusion now follows by a generalization to complete metric spaces (like Δ) of a simple argument due to Carl Neumann and often exploited by Picard, which is purely geometrical.

COROLLARY 1: $\sup pT^n \leq p + \sum_{k=0}^{\infty} (pT^{k+1} - pT^k)$ is finite, for any fixed p . (Cf. §2, conclusion (11)).

COROLLARY 2: Let T_1, \dots, T_n be any sequence of transition operators, and let d_i denote $\inf_{p \in \Delta} pT_i$. Then for all p, q in Δ , $\eta(pT_1 \dots T_n - qT_1 \dots T_n) \leq 2 \prod_{i=1}^n (1 - |d_i|)$.

6. *Ergodic Hypothesis*.—Unless the conclusion of Corollary 1 holds, the means of the pT^k at best tend to 0. Hence we shall fix p , and make the

ERGODIC HYPOTHESIS: The pT^k have an upper bound. This is fulfilled if p is the integral of a bounded density-function, and T leaves measure invariant: the integral of the upper bound to the density function is an upper bound to the pT^k .

THEOREM 2: The Ergodic Hypothesis implies the existence of at least one stable distribution.

Proof: Form $h = \limsup_{k \rightarrow \infty} pT^k = \inf_n \sup_{k \geq n} pT^k$; evidently $hT = T$, $|h| \geq 1$, and so $h > 0$. Hence $h/|h|$ is a stable distribution.

THEOREM 3 (mean ergodic theorem): The Ergodic Hypothesis implies that the means $\frac{1}{n} \sum_{k=0}^{n-1} pT^k$ converge weakly, in the sense that if $\lambda(f)$ is any linear functional, then the numerical means $\phi_n(p) = \lambda\left(\frac{1}{n} \sum_{k=0}^{n-1} pT^k\right)$ converge in the ordinary sense.

Proof: By a generalization of a Lemma of Hahn (cf. §7), λ is the sum of its positive and negative parts. Again, by a simple Lemma of Banach

(op. cit., p. 54) each part is a constant multiple of a functional satisfying $0 \leq \lambda(f) \leq \eta(f)$ for all $f > 0$. Hence we need only consider this case. Again, if k is large, then $h = \limsup_{k \rightarrow \infty} pT^k$ contains an arbitrarily large part of pT^k , together with all transforms of this part; hence we can assume $p \leq h$, where $hT = h$.

We shall make these assumptions, and in the proof, shall treat all "parts" f of h on the same footing. First, define $\bar{\phi}(f) = \limsup_{n \rightarrow \infty} \phi_n(f)$, $\underline{\phi}(f) = \liminf_{n \rightarrow \infty} \phi_n(f)$. Clearly $0 \leq \underline{\phi}(f) \leq \bar{\phi}(f) \leq \eta(f)$; clearly also $\underline{\phi}(fT) = \underline{\phi}(f)$ and $\bar{\phi}(fT) = \bar{\phi}(f)$. The functionals $\underline{\phi}$ and $\bar{\phi}$ are monotone; they need not be linear, but $\underline{\phi}$ is convex while $\bar{\phi}$ is concave. Hence the functionals (we use a construction of F. Riesz)

$$(\bar{\alpha}f) = \sup \sum_i \bar{\phi}(f_i) \qquad \underline{\alpha}(f) = \inf \sum_i \underline{\phi}(f_i)$$

where the summations are with respect to all decompositions of f into (finite or countable; it makes no difference) parts f_i , are, respectively, the least linear functional $\geq \bar{\phi}$, and the greatest linear functional $\leq \underline{\phi}$. Moreover $\bar{\alpha}(fT) = \bar{\alpha}(f)$ and $\underline{\alpha}(fT) = \underline{\alpha}(f)$; the functionals are invariant.

Now since $0 \leq \underline{\alpha} \leq \underline{\phi} \leq \bar{\phi} \leq \bar{\alpha} \leq \eta$, and $h \geq f$, in order to conclude $\underline{\phi}(f) = \bar{\phi}(f) = \lim_{n \rightarrow \infty} \phi_n(f)$, we need only show that $\underline{\alpha}(f) = \bar{\alpha}(f)$, whence, since $\bar{\alpha} - \underline{\alpha}$ is non-negative and linear, we need only show $\bar{\alpha}(h) \leq \lambda(h) \leq \underline{\alpha}(h)$. By duality, we need only show $\bar{\alpha}(h) \leq \lambda(h)$. This is just what we shall prove.

7. *Extension of a Lemma of Hahn.*—For it, we shall need an extension of a Lemma of Hahn.⁶ Let $\lambda(x)$ be any linear functional on Σ , let Λ^+ denote the set of $u > 0$ such that $0 < x \leq u$ implies $\lambda(x) > 0$, and define Λ^- dually. Further, let Λ^0 denote the set of $u > 0$ such that $0 < x \leq u$ implies $\lambda(x) = 0$.

LEMMA: Σ is decomposed into three components: a component Λ^+ on which $x > 0$ implies $\lambda(x) > 0$, a component Λ^0 on which $\lambda(x) = 0$, and a component Λ^- on which $x > 0$ implies $\lambda(x) < 0$.

COROLLARY 1: Any linear functional can be resolved into its positive part and its negative part.

8. *Completion of Proof.*—Choose $\epsilon > 0$, and denote by g_n the component of h (cf. §7) on which $\phi_n(x) - \bar{\alpha}(x) + \epsilon\eta(x)$ is non-negative. Then irrespective of ϵ ,

LEMMA: The join u of the g_n is h .

Proof: Consider $r = h - u$; evidently for all n , $r \leq h - g_n$. Hence $\bar{\phi}(x) \leq \sup \phi_n(x) \leq \bar{\alpha}(x) - \epsilon\eta(x)$ for all $x \leq r$, and so $\bar{\alpha}(r) = \sup \sum \bar{\phi}(x_i) \leq \bar{\alpha}(r) - \epsilon\eta(r)$, whence $\eta(r) = 0$ and $r = 0$.

Now let h_n denote the part of g_n not in $g_1 \cup \dots \cup g_{n-1}$; the h_n are the components of a direct decomposition of h . Choose M so large that if h^* denotes $h - \sum_{k=1}^m h_k$, then $|h^*| < \epsilon$. We shall show that for all $N > M$,

$$(E) \quad N\lambda(h) \geq N\bar{\alpha}(h) - N\epsilon\eta(h) - N\epsilon - M\bar{\alpha}(h)$$

from which, dividing through by N , and letting $N \rightarrow \infty$, we will get $\lambda(h) \geq \bar{\alpha}(h) - \epsilon\eta(h) - \epsilon$. Now letting $\epsilon \rightarrow 0$, the proof is complete.

The proof of (E) reproduces the combinatorial essence of G. D. Birkhoff's proof of the Strong Ergodic Theorem.² We rely on the fact that the "components" of h form a Boolean algebra, and may be treated like sets.

¹ L. Kantorovitch, "Lineare halbgeordnete Raume," *Math. Sbornik*, 2, 121-68 (1937). Cf. also H. Freudenthal, "Teilweise geordnete Moduln," *Proc. Akad. Wet. Amsterdam*, 39, 641-51 (1936).

² J. von Neumann, "Proof of the Quasi-Ergodic Hypothesis," these PROCEEDINGS, 18, 70-82 (1932). Our method is that used by G. D. Birkhoff for his stronger result; cf. "Proof of a Recurrence Theorem for Strongly Transitive Systems, and Proof of the Ergodic Theorem," these PROCEEDINGS, 17, 650-60 (1931).

³ In the sense of the author's "On the combination of subalgebras," *Proc. Camb. Phil. Soc.*, 29, 441-64 (1933). Synonyms are "Verband" (Fr. Klein) and "structure" (O. Ore). We shall use the notation $f \vee g$ for $\sup(f, g)$ and $f \wedge g$ for $\inf(f, g)$.

⁴ S. Banach, "Théorie des opérations linéaires," Warsaw, 1933. By general consent, the "B-spaces" of Banach, op. cit., are called Banach spaces; they are complete, metric, linear spaces.

⁵ In the sense of G. D. Birkhoff and Paul Smith, "Structure Analysis of Surface Transformation," *Jour. Math.*, 7, 365 (1928).

⁶ The author is much indebted to J. von Neumann for suggesting that this lemma could be generalized. He is also indebted to S. Ulam for many conversations on the whole subject.

ON SEMI-GROUPS OF TRANSFORMATIONS IN HILBERT SPACE

BY EINAR HILLE

DEPARTMENT OF MATHEMATICS, YALE UNIVERSITY

Communicated February 9, 1938

1. Let E be a normed complete linear vector space, in other words a space (B) in the terminology of Banach. Let $x \in E$ and let $T_\alpha(x)$ be a linear transformation on E to E defined for every $\alpha > 0$. If

$$T_\alpha(T_\beta(x)) = T_{\alpha + \beta}(x), \quad (1)$$

we say that $\{T_\alpha(x)\}$ forms a *semi-group*.

Assuming in addition that

$$\|T_\alpha(x)\| \leq \|x\|, \quad (2)$$

I have investigated some of the properties of these transformations.¹ Continuing this study, I have found the case in which E is a Hilbert space and $T_\alpha(x)$ is a self-adjoint, positive definite transformation of particular

interest. In this case there exists a representation of $T_\alpha(x)$ analogous to that found by M. H. Stone for unitary transformations forming a group.²

2. The main result is the following theorem.

Let $T_\alpha(x)$, $\alpha > 0$, be a family of self-adjoint, positive definite transformations on \mathfrak{H} to \mathfrak{H} satisfying (1) and (2). Then there exists a self-adjoint transformation $A(x)$, positive definite but not necessarily bounded, with its resolution of the identity $E(\lambda)$, such that

$$(T_\alpha(x), x) = \int_0^\infty e^{-\alpha\lambda} d_\lambda(E(\lambda)x, x). \quad (3)$$

It follows in particular that for a fixed x , $(T_\alpha(x), x)$ is an analytic function of α , holomorphic for $\Re(\alpha) > 0$ and continuous for $\Re(\alpha) \geq 0$. This would seem to be the reason why the proof can be carried through without any assumptions regarding the continuity or measurability of $(T_\alpha(x), x)$.

A detailed proof will be published elsewhere, but the following outline of the argument will probably be found sufficient to enable the interested reader to fill in the omissions. The basic observation is that

$$\Delta_h^n(T_\alpha(x), x) = \sum_{k=0}^n (-1)^k \binom{n}{k} (T_{\alpha + kh}(x), x) \geq 0$$

for every $\alpha \geq 0$, $h > 0$, $n \geq 0$. Hence $(T_\alpha(x), x)$ is completely monotone in $0 \leq \alpha < \infty$ and by the Bernstein-Widder theorem³

$$(T_\alpha(x), x) = \int_0^\infty e^{-\alpha\lambda} dV(\lambda; x), \quad (4)$$

where $V(\lambda; x)$ is a never decreasing function of λ and $V(0; x) = 0$. By (2) $0 \leq (T_\alpha(x), x) \leq (x, x)$ so that $V(\lambda; x) \leq (x, x)$.

The real inversion formulas for the Laplace integral show that $V(\lambda; x)$ is a linear functional in $(T_\alpha(x), x)$. The latter being bilinear in x , we conclude the existence of a bilinear functional $V(\lambda; x, y)$ such that

$$(T_\alpha(x), y) = \int_0^\infty e^{-\alpha\lambda} dV(\lambda; x, y), \quad (5)$$

and $V(\lambda; x, x) = V(\lambda; x)$. Further

$$|V(\lambda; x, y)|^2 \leq (x, x) (y, y),$$

whence it follows that $V(\lambda; x, y) = (E(\lambda)x, y)$. The transformations $E(\lambda)x$ are evidently self-adjoint. Replacing x by $E(\mu)x$ in (5) and putting $\alpha = 0$, we easily conclude that $E(\lambda)x$ is a resolution of the identity. We then define the corresponding self-adjoint transformation $A(x)$ in the usual manner by

$$(A(x), y) = \int_0^\infty \lambda d_\lambda(E(\lambda)x, y),$$

the domain of $A(x)$ being that subset of \mathfrak{S} for which

$$\int_0^\infty \lambda^2 d_\lambda(E(\lambda)x, x) < \infty.$$

That $A(x)$ is positive definite follows from the fact that $E(\lambda) = 0$ for $\lambda \leq 0$. Incidentally we observe that it is permitted to interpret $A(x)$ as a determination of $-\log T_1(x)$.

3. Among the various transformations forming semi-groups we select the Poisson integral for the half-plane, i.e.,

$$P_\alpha(f) = \frac{\alpha}{\pi} \int_{-\infty}^{\infty} \frac{f(t+u)}{u^2 + \alpha^2} du. \quad (6)$$

If $f(t) \in L_2(-\infty, \infty)$ so does $P_\alpha(f)$ and conditions (1) and (2) are satisfied. Moreover, $P_\alpha(f)$ is a self-adjoint, positive definite transformation. A straightforward calculation gives

$$(P_\alpha(f), f) = \int_{-\infty}^{\infty} e^{-\alpha|\lambda|} |F(\lambda)|^2 d\lambda,$$

where $F(\lambda)$ is the Fourier transform of $f(t)$. Putting

$$D_\lambda(f) = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{\sin \lambda u}{u} f(t+u) du \quad (7)$$

for $\lambda > 0$, $D_\lambda(f) = 0$ for $\lambda \leq 0$, and utilizing the relation

$$(D_\lambda(f), f) = (D_\lambda(f), D_\lambda(f)) = \int_{-\lambda}^{\lambda} |F(u)|^2 du, \lambda > 0,$$

we find

$$E(\lambda)f = D_\lambda(f), A(f) = \tilde{f}', \quad (8)$$

where $\tilde{g}(t)$ is the conjugate function of $g(t)$.

¹ E. Hille, "Notes on Linear Transformations. I," *Trans. Amer. Math. Soc.*, **39**, 131-153 (1936).

² M. H. Stone, "Linear Transformations in Hilbert Space," these PROCEEDINGS, **16**, 172-175 (1930), and "On One-Parameter Unitary Groups in Hilbert Space," *Ann. Math. (2)* **33**, 643-648 (1932). See also J. von Neumann, "Über einen Satz von M. H. Stone," *Ann. Math., Ibid.*, 567-573, and F. Riesz, "Über Satze von Stone und Bochner," *Acta Szeged*, **6**, 184-198 (1933).

³ S. Bernstein, "Sur les fonctions absolument monotones," *Acta Math.*, **52**, 1-66 (1929). D. V. Widder, "Necessary and Sufficient Conditions for the Representation of a Function as a Laplace Integral," *Trans. Amer. Math. Soc.*, **33**, 851-892 (1931).

⁴ See loc. cit., note¹ for the properties of $P_\alpha(f)$ and $D_\lambda(f)$.

RESIDUATED LATTICES

BY MORGAN WARD AND R. P. DILWORTH

DEPARTMENT OF MATHEMATICS, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated February 14, 1938

1. *Introduction.*—We summarize here our investigations of a lattice $\Sigma: a, b, \dots, z$ over which a multiplication or a residuation is defined. (Ward 1, Dilworth 1.) We denote division, union and cross-cut by $x \supset y$, (x, y) , $[x, y]$. Σ is closed with respect to union (cross-cut) if any set of elements have a union (cross-cut). The unit and null elements i and n are defined by $i \supset x$, $x \supset n$, every $x.a$ covers b (Birkhoff 1) if $a \supset b$, $a \not\supset b$ and $a \supset x \supset b$ implies $x = a$ or $x = b$. Elements covered by i are called divisor-free. A sub-lattice Λ is dense over Σ if Λ contains l, m and $l \supset x \supset m$ imply Λ contains x . An element a is a node if either $x \supset a$ or $a \supset x$, every x . Any involution of Σ interchanging union and cross-cut is called a negation. An element a is idempotent relative to a binary operation $x \circ y$ if $a \circ a = a$. Two properties P and Q which Σ may possess are completely independent if there exist instances of lattices in which both P and Q hold, neither holds, P holds but not Q , Q holds but not P .

2. *Residuations and Multiplications.*—Assume Σ contains i . A well defined binary operation $x:y$ is called a residuation over Σ provided that

- R 1. $a:b$ lies in Σ whenever a, b lie in Σ .
- R 2. $a:b = i$ if and only if $a \supset b$.
- R 3. $a \supset b$ implies $a:c \supset b:c$ and $c:b \supset c:a$.
- R 4. $(a:b):c = (a:c):b$.
- R 5. $[a, b]:c = [a:c, b:c]$ and $c:(a, b) = [c:a, c:b]$.

This residual has the formal properties of the residual in polynomial ideal theory. (Ward 1, Dilworth 1.)

THEOREM. *A residuated lattice closed with respect to cross-cut is also closed with respect to a well-defined multiplication $x \cdot y$ satisfying the following conditions:*

- M 1. $a \cdot b$ lies in Σ whenever a, b lie in Σ .
- M 2. $(a \cdot b) \cdot c = a \cdot (b \cdot c)$.
- M 3. $a \cdot b = b \cdot a$.
- M 4. $a \cdot i = a$.
- M 5. $a \cdot (b, c) = (a \cdot b, a \cdot c)$.

If a multiplication over Σ satisfies M 1–M 5 and M 6: *The product of the unions of any two sets of elements of Σ is the union of the products of all pairs of elements of the sets*, then a residuation exists satisfying R 1–R 5. (Ward 1.) The relationship between the two operations is as follows.

$a \supset (a:b) \cdot b$; if $a \supset x \cdot b$ then $a:b \supset x$.
 $(a \cdot b):a \supset b$; if $x:a \supset b$ then $x \supset a \cdot b$.

Both operations may be dualized.

THEOREM. *The Dedekind modular condition and the existence of a residual are completely independent properties of a lattice. The existence of a residual and the existence of a negation are completely independent.*

3. Conditions for Residuation.

THEOREM. *Every distributive lattice which is closed with respect to union can be residuated in at least one way. (Ward 2.)*

THEOREM. *Every Boolean algebra can be residuated in only one way.*

The residual in this case is $a:b = a \vee b'$. (Dilworth 1.) A lattice is said to be complemented (Birkhoff 2) if it contains i and n and for every element a an element a' such that $(a, a') = i$, $[a, a'] = n$.

THEOREM. *The only complemented lattices which can be residuated are Boolean algebras.*

COROLLARY. *No non-trivial projective geometry (Birkhoff 2) can be residuated.*

THEOREM. *The free modular lattice of order twenty-eight cannot be residuated.*

THEOREM. *Every lattice in which only one divisor free element exists can be residuated in at least one way.*

THEOREM. *A lattice built up out of a set of residuated lattices connected into a chain by nodes can be residuated.*

THEOREM. *A direct product of residuated lattices can be residuated; conversely if a residuated lattice can be expressed as a direct product, each of its factors can be residuated.*

THEOREM. *A necessary condition that a residuated lattice in which an ascending chain condition holds (Ore 1) can be residuated is that every Boolean algebra generated by a finite number of divisor free elements be dense over the lattice.*

4. *Noether Lattices.*—We propose here the name "Noether lattice" for any residuated modular lattice in which both the (ascending) chain condition holds and

D 1. *For any two elements a, b of Σ , there exist exponents r and s such that $a \cdot b \supset [a^r, b^s]$.*

For the ideal theory terminology used here see van der Waerden 1.

THEOREM. *In a Noether lattice, every irreducible is primary. Conversely, if in a residuated modular lattice with chain condition every irreducible is primary, then condition D 1 holds.*

THEOREM. *The three decomposition theorems and the uniqueness theorems of E. Noether for the ideals of a commutative ring in which the chain condition holds are all valid in an abstract Noether lattice.*

THEOREM. *Condition D 1 is completely independent both of the modular condition, the distributive condition and the chain condition.*

THEOREM. *A necessary and sufficient condition that a finite residuated modular lattice be a Noether lattice is that $a \cdot b = [a, b]$ for all idempotent elements a, b of the lattice.*

THEOREM. *A sufficient condition that a residuated modular lattice in which the ascending chain condition holds be a Noether lattice is*

$$M\ 7. \quad a \cdot [b, c] = [a \cdot b, a \cdot c].$$

The resulting lattice need not be distributive.

5. *Distributive Residuated Lattices.*—Consider a lattice in which one or more of the following conditions hold:

D 2. *If $a \supset b$, there exists at least one element q such that $a \cdot q = b$.*

R 6. $(a:b, b:a) = i$.

R 7. $a:[b, c] = (a:b, a:c)$.

R 8. $(b, c):a = (b:a, c:a)$.

THEOREM. *Every lattice closed with respect to union in which D 2 holds can be residuated, and is distributive.*

THEOREM. *If Σ is a residuated lattice, any one of R 6, R 7, R 8 implies Σ is distributive. R 6 and D 2 implies R 7 and R 8. R 8 implies R 7.*

We call a residuated lattice satisfying D 2 and R 6 *semi-arithmetical*. The properties of such lattices are similar to the instance in Ward 2 where multiplication is cross-cut.

6. *Residuated Group Lattices—Dual Operations.*—On assuming Σ is a semi-group and D_2 , we may pass to the group Γ of quotients a/b . We have made Γ into a residuated lattice having properties R 1–R 8. However the lattice has no unit element.

The existence of a dual residuation and multiplication is completely independent of the existence of the initial residuation and multiplication. An interesting case arises in a residuated lattice containing n if the correspondence $a \rightarrow n:a$ is a negation. The lattice is then distributive, and multiplication and its dual are also distributive with respect to one another.

Proofs of these results will be published elsewhere.

G. Birkhoff: 1. *Proc. Cambridge Phil. Soc.*, 29, 441–464 (1933).

2. *Ann. Math.* (2), 36, 743–748 (1935).

R. P. Dilworth: 1. *Bull. Am. Math. Soc.*, 44, April (1938).

O. Ore: 1. *Ann. Math.*, (2), 36, 406–437 (1935).

B. L. van der Waerden: 1. *Moderne Algebra*, 2, Berlin (1931).

Morgan Ward: 1. *Duke Jour.*, 3, 627–636 December (1937).

2. "Structure Residuation." Submitted to the *Ann. Math.*

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

April 15, 1938

Number 4

Copyright 1938 by The National Academy of Sciences

INTERSEXUALITY IN *DROSOPHILA VIRILIS* AND ITS BEARING
ON SEX DETERMINATION

BY G. A. LEBEDEFF

DEPARTMENT OF PLANT BREEDING, CORNELL UNIVERSITY

Communicated March 14, 1938

It is a well-established fact that in higher animals and plants individuals of either sex possess both female and male tendencies. The superiority of the one tendency over the other determines the sex.

There are two principal theories as to the balance between sex tendencies: qualitative of Bridges, and quantitative of Goldschmidt. These two theories have been subjected to criticism and to some modification. A review of the literature and the bibliography will be given in a detailed account of this work to be published later. For the present it is sufficient to mention two shortcomings of these theories.

1. In the experiments noted no sex gene has been isolated, and therefore the balance between female and male tendencies has been studied only indirectly.

2. In these studies, the female and male tendencies have been considered as alternative, and therefore these theories do not explain the nature of hermaphroditism.

The present author for the last several years has been engaged in the study of intersexuality in *Drosophila virilis*. In the course of this study facts concerning sex expression in this species have been discovered which throw new light on the mechanism of sex determination. The hypothesis here presented is the result of these studies. The progress of this work has been reported before meetings of the Genetics Society of America (Lebedeff, 1934 and 1938). The author wishes to express his sincere appreciation to Professors R. A. Emerson and L. W. Sharp of Cornell University for their advice and criticism.

Several years ago in one of the *D. virilis* stocks a new mutant was discovered. The gene concerned was called intersex (*ix*)* and was found to be located in the third chromosome, the exact locus being determined. The gene has no effect on males, but females homozygous *ix^m/ix^m* become converted into males which are sterile. In the course of this work two

modifying genes (designated *A* and *B*) have been found which delay the transformation of these females into males, thereby bringing about the development of hermaphrodites. The genetical evidence indicates that the number of intersex modifying genes is probably greater than two and that their effect is cumulative. The degree of hermaphroditism varies from female-like to nearly male-like. The specimens intermediate between these two extremes possess well-developed female and male sexual characteristics, including external and internal sexual organs and gonads. However, they are sterile, since gametogenesis is abortive.

Genetical and morphological studies of these intersexes, supplemented by embryological studies of gonads, indicate that the Time Law of Intersexuality found to be responsible for intersexuality in *Lymantria* and *Drosophila melanogaster* applies to *D. virilis* intersexes also. The intersexes of *D. virilis* start to develop as females and then from a certain stage, known as the turning point, proceed to develop in a male direction. The sexual organs whose imaginal discs are differentiated before the turning point is reached develop into female organs. On the other hand, the imaginal discs which appear after the occurrence of the turning point develop in the adult intersex into male organs. Thus, in *D. virilis*, as in the other species mentioned, the time of the reversal determines the degree of intersexuality. Nevertheless, intersexes of *D. virilis* differ morphologically from all other types, being essentially hermaphrodites because of the peculiarity in the development of those organs whose imaginal discs had not become fully differentiated at the time of reversal. Such imaginal discs develop into female organs, but along with them male organs appear, presumably from fresh outpushings. The two systems, the original female and the additional male, develop side by side, resulting in hermaphroditism.

The study of the development of gonads in these intersexes indicates that in all morphological types of intersexes the gonads start their development as ovaries. In the female type of intersexes the ovaries continue their development, though it is retarded in various degrees. In the hermaphrodites, after the occurrence of the "turning point" the female germinal cells in the ovaries are gradually transformed into male-like ones, while the ovary buds out a testis-like structure to which migrate some of the oocytes, now transformed into spermatocyte-like cells. In the male type of intersexes the ovaries are gradually transformed into testis-like organs, in which the cells undergo a process of transformation into spermatocyte-like cells, most of which eventually disintegrate.

These findings are of primary theoretical importance. According to the prevalent theories, the development of an individual at any given time is under the control of either the male or the female tendency, depending on the quantitative or qualitative balance between the two. These two

tendencies are considered as alternative. Although this assumption is obviously contradictory to the state of hermaphroditism, it has been retained in most theories, because our understanding of hermaphroditism was entirely unsatisfactory. "Our present conception of hermaphroditism is the most unsatisfactory chapter in the whole sex problem, and our material is insufficient to permit of a correct genetic or physiological understanding." (Goldschmidt, 1923). However, our own studies indicate that the development of an individual can proceed under the direct and partially simultaneous influence of tendencies of both sexes, resulting in the development of hermaphrodites. It is hoped that the problem discussed here may improve our understanding of the genetics and the physiology of hermaphroditism.

We are now prepared to ask what is the bearing of this study on the mechanism of sex determination? To answer this question we should first clarify our concept of the ix^m gene and its normal allele.

Goldschmidt (1935) and Patterson, Stone and Bedichek (1937) ventured to deal with these questions. Their conclusions were based on the preliminary report of our work (Lebedeff, 1934). Goldschmidt considers the ix^m gene as an autosomal male determiner, the potency of which is not strong enough to overbalance the female tendencies of the 2X:2A mechanism. The ix^m gene is compared to weak M of *Lymantria*. Patterson and his co-workers also considered the ix^m gene as a gene for maleness. But its normal allele is considered to be the gene for femaleness.

Our experimental data, especially those unknown to the above mentioned authors, indicate that these concepts of the ix^m gene and its normal allele are incorrect. According to our conception, the normal allele of the ix^m gene is a normal male determiner (Ix^m), and in the 2X:2A individuals it is in a balanced condition. The mutant ix^m is considered to be a strong allele of Ix^m . The potency of this gene is so great that it partially overrides the 2X:2A mechanism, resulting in the conversion of females homozygous for this gene into males which are sterile. The discovery of this gene is considered by Patterson and his co-workers as the nearest approach to a demonstration of the action of a single gene in sex determination in *Drosophila*. To this we may add that this discovery permits for the first time a more direct approach to the rôle of a balance between female and male determining genes in the process of sex determination.

It is a well-established fact that in *Drosophila* the X chromosome is the carrier of the gene (or genes) for femaleness. Without going into a detailed discussion of this question, which has aroused controversy in the last few years, we may assume, probably to the satisfaction of a large group of geneticists, that the theory of a single female gene in the X chromosome in *Drosophila* is the more probable. Accordingly, the normal *Drosophila* females are homozygous for this gene (FF), and the males

have a single dose of it (F). To this genotype we shall now add the gene for maleness (Ix^m) located in the third chromosome of *D. virilis*. The normal females then will be $FF Ix^m Ix^m$ and the normal males $F Ix^m Ix^m$.

It is a simple matter to explain why $F Ix^m Ix^m$ animals develop into normal males, assuming that two doses of Ix^m are more potent than one dose of F . But it is not so easy to explain why $FF Ix^m Ix^m$ individuals develop into normal females. According to Goldschmidt's theory, the quantitative difference between F and M shifts the balance into one or another direction. According to our hypothesis both M and F factors are equally potent in determining whether the animal shall develop into a female or a male, but there is a special mechanism which decides which of these factors will function in the zygote. This mechanism either inhibits the activity of the F factor, thus giving to the M factor full control in determining sex, with the consequent development of normal males; or the mechanism inhibits the activity of the M factor, thus shifting the control of sex development to the F factor, resulting in the development of normal females.

TABLE 1

Phenotypical expression of the 2X:2A individuals in regard to the ix^m gene with its modifiers and suppressors. To simplify the scheme suppressors (S_1 and S) and modifier (B) are used in a heterozygous condition only. For the same reason only one modifier is considered here.

Any one of these genotypes when in a 1X:2A individual results in the development of normal males.

We have found such a mechanism in *D. virilis*. Two dominant suppressors were found, either one of which entirely inhibits the action of the ix^m gene (table 1). Therefore, 2X:2A individuals homozygous for ix^m and homo- or heterozygous for both or either of these suppressors are not converted into sterile males but develop into normal females. One of these suppressors, designated as S_1 , is located in the third chromosome, and therefore is linked with ix^m and its normal allele Ix^m . The other suppressor, designated as S , belongs to one of the other autosomes. These suppressors probably are not sex genes, and are considered as neutral. However, they play an important rôle in the mechanism of sex determination in that they can inhibit the action of Ix^m , thus determining the direction in which the development will proceed.

Now we shall add these suppressors to the above outlined formulae for male and female. The $FF (Ix^m Ix^m S_1 S_1) SS$ is a female because the development of the male tendency is inhibited by S_1 and S suppressors. The $F (Ix^m Ix^m S_1 S_1) SS$ individuals develop into males in spite of the presence of both suppressors of maleness, because two doses of Ix^m are opposed by only one dose of F . Under such conditions the suppressors

are unable to inhibit completely the action of Ix^m , hence, the balance goes in a male direction.

To evaluate further the nature of the ix^m gene and its suppressors let us make one assumption, namely, that male-like intersexes (which often resemble normal males in all features) might become fertile. This might result either from a mutation of the ix^m gene into its stronger allele, or from the appearance of a new mutant, a specifically female suppressor (S_f). In the former case it would probably result in a mechanism comparable to that of *Lymantria*, and in the latter case it would lead to the establishment of the WZ mechanism of sex determination. Then the cross between an $ix^m/+$ female and an ix^m/ix^m male-like intersex would result in a female and male population in a ratio of 1:1. But both females and males from this cross would be of 2X:2A constitution. Thus, a new race of *D. virilis* would be established in which the XY mechanism is displaced by the WZ, since females would be heterozygous and males homozygous for the sex chromosome. The third chromosome, in which the ix^m gene is located, would have assumed the rôle of the sex chromosome. The males in this hypothetical case would be genotypically $FF ix^m ix^m S_f S_f$, and the females $FF Ix^m ix^m S_f S_f$ in constitution. In this case F is homozygous in both sexes, and ix^m is homozygous in males and heterozygous in females, and S_f suppresses F activity in individuals homozygous for both F and ix^m factors.

Thus, XY and WZ mechanisms are visualized as the factors controlling the activity of the suppressors. In the *Drosophila* XY type the suppressors inhibit the activity of the M gene, and for that reason $FF (MM S_1 S_1) SS$ animals develop into females. In species with the WZ mechanism suppressors inhibit the female tendency, both sexes probably being homozygous for FF . In unisexual species in which neither the XY nor the WZ mechanism is known, the effectiveness of suppressors is probably influenced by the heterozygosity of either F or M factors. This condition is less balanced, the specificity of the suppressors may be undifferentiated and the development may proceed under the influence of both F and M genes, resulting in hermaphroditism. In bisexual species all individuals are assumed to be homozygous for both F and M factors, and therefore suppressors are not effective.

It is evident from the data here presented that hermaphroditism may occur only in the sex homozygous for both F and M factors. In species with the XY mechanism hermaphrodites develop when the M factor mutates to its stronger allele, as has been demonstrated in our studies. In the species with the WZ mechanism hermaphrodites probably will develop when the F factor mutates to a stronger allele. In the species in which there is no sex chromosome both sexes are homozygous for both F and M factors and hermaphroditism is therefore of common occurrence.

The experiments here briefly described are essentially a genetical and physiological study of the sex determining mechanism in *Drosophila* in the process of transformation of a normal unisexual into a hermaphroditic (though non-functional) state. In connection with this study the experiments of Emerson (1932) and Jones (1934) with maize are of considerable interest. These authors independently described the genetics of the transformation of normal monoecism into dioecism.

Normally, maize plants are monoecious, the terminal inflorescence bearing staminate and the lateral bearing pistillate flowers. In the inflorescence of each sex, rudiments of the opposite sex are usually present. Numerous genes, mostly recessive, have been found which influence the expression of sex. These genes are assumed to stimulate a certain potentiality that is usually dormant, and suppress the other character normally functioning. By a suitable combination of these genes strains were obtained which produced only a pistillate or a staminate inflorescence. These genes are considered as a few of numerous sex genes, the interaction of which is responsible for monoecism in this species.

Our experiments with *D. virilis* hermaphrodites permit a further evaluation of this work. According to our hypothesis, in monoecious species both sexes are homozygous for both *M* and *F* factors, and therefore are free of specifically *M* or *F* suppressors. There is, however, some mechanism, which was also assumed by Jones, for the strict localization of flowers of both sexes on different parts of the plant. In maize, staminate flowers occupy the terminal position, and pistillate flowers the lateral position.

All known genes in this species, which affect flower expression, can be classified into several groups according to the effect they produce. Some of them inhibit the development of the staminate flowers in their normal terminal position. They are assumed to be male (*M*) suppressors. To this group belong numerous so-called male-sterile mutants. While the genes which inhibit the development of the pistillate flowers in their normal lateral position are assumed to be female (*F*) suppressors, to which such mutants as barren-stalks and silkless belong.

Besides these suppressors, which inhibit the development of either male or female flowers in their respective normal position on the plant, there are a number of genes which stimulate the development of male or female flowers in places where they are usually in a very rudimentary state. These genes are assumed to be male (*M*) or female (*F*) stimulating genes, or male and female "exciters" (Aida's terminology). The development of usually dormant stamens in the lateral inflorescence is assumed to be due to male exciting genes, to which such mutants as dwarfs and anther ear belong; while the development of usually dormant pistils in the terminal inflorescence is assumed to be a result of female exciters, to which such mutants as tassel-seed 4 and 5, and silky ears belong.

Several known mutants in maize seem to have a double effect, possibly due to the fact that two or more genes are closely linked. To this series belong such mutants as tassel-seed 1, 2 and 3, which are both male suppressors and female exciters.

In our discussion of the mechanism of sex determination the rôle played by the sex modifying genes has not been touched. The presence of these genes can be detected only in the intersexes. Bridges (1932) and Dobzhansky and Schultz (1934) have demonstrated that the female sex modifying genes in *D. melanogaster* are preponderantly located in the X chromosome. In this study two such genes have been demonstrated in *D. virilis*. The available evidence indicates that the number of such genes is probably greater than two and that their effect is cumulative. Their function is to fix the time of the turning point, i.e., to determine the extent of the development of one sex before the control of development is shifted to give the opposite sex. The activity of these genes can be easily influenced by internal and external environment.

It is clear that the sex modifying genes (which properly should be called intersex modifying genes) play an important rôle in the development of a given sex. Recently much attention has been given to the question of the rôle played by these genes in the sex determining mechanism. The Pasadena group of geneticists led by Bridges, Dobzhansky and Schultz, consider these genes as the sex determining genes. Their opponents, Goldschmidt, Patterson and co-workers consider that the presence of these genes has not been sufficiently demonstrated.

The present author is inclined to take an intermediate position in this discussion. The presence of the intersex modifying genes cannot be disputed, but the rôle played by them in the development of normal males and females is debatable. As intersex modifying genes they are involved in the development of sex. But in the complex development of a given sex two processes can be recognized: sex determination and sex differentiation (Baltzer, Goldschmidt, Witschi). The so-called sex modifying (or intersex modifying) genes, according to our hypothesis, are the sex differentiating genes.

Ascribing to intersex modifying genes the rôle of sex differentiation, we naturally should provide an explanation of triploid intersexuality, in connection with which these genes were first detected. According to our hypothesis, triploid intersexes of *D. melanogaster* are of $FF (Ix^m Ix^m Ix^m S_1 S_2 S_3) SSS$ constitution. It is obvious that the initial balance is on the male side, so that these individuals should start their development as males. The balance, however, is not stable and intersexuality results. It is also possible on the basis of our study to explain why haploid *Drosophila* $F (M S_1) S$ is a female.

Our conception of the mechanism of sex determination and differentia-

tion in *D. virilis* may be summarized as follows. Sex in this species is determined by the balance between the gene for femaleness (F) located in the X chromosome, and the gene for maleness (Ix^m) located in the third chromosome. These factors are equally potent in determining whether the animal will develop into a female or a male. The balance between these genes is maintained by a set of suppressors, which inhibit the activity of the Ix^m gene when it is opposed by an equal dose of F genes. For that reason $FF (Ix^m Ix^m S_2 S_2) SS$ individuals develop into females. The $F (Ix^m Ix^m S_2 S_2) SS$ individuals develop into males in spite of the presence of the Ix^m suppressors, because the Ix^m factors are not counter-balanced by an equal dose of the F factors. The XY mechanism is visualized as the factor controlling the activity of the suppressors, which are specifically suppressors of maleness.

The conception outlined here is not considered final. It is hoped that further research with *D. virilis* intersexes and with the sex genes of maize will help in evaluating the hypothesis here outlined.

$\frac{ix^m S_2}{ix^m +} S/+ B/+$ female	$\frac{ix^m +}{ix^m +} S/+ B/+$ female
$\frac{ix^m S_2}{ix^m +} S/+ +/+$ female	$\frac{ix^m +}{ix^m +} S/+ +/+$ female
$\frac{ix^m S_2}{ix^m +} +/+ B/+$ female	$\frac{ix^m +}{ix^m +} +/+ B/+$ hermaphrodite
$\frac{ix^m S_2}{ix^m +} +/+ +/+$ female	$\frac{ix^m +}{ix^m +} +/+ +/+$ sterile male

* The gene was called intersex (ix) soon after it had been discovered, and before its nature had been studied. Now we know that this gene is the gene for maleness, and therefore it will be called ix^m and its normal allele Ix^m .

THE SEDIMENTATION CONSTANT OF VISUAL PURPLE*

BY SELIG HECHT AND E. G. PICKELS

LABORATORY OF BIOPHYSICS, COLUMBIA UNIVERSITY, AND LABORATORIES OF THE INTERNATIONAL HEALTH DIVISION, ROCKEFELLER FOUNDATION, NEW YORK

Communicated March 9, 1938

1. Visual purple, the photosensitive substance of the vertebrate rods, is most likely a protein of high molecular weight.¹ We have determined its sedimentation constant under several conditions in order to answer some questions with regard to its absorption spectrum, its size and chemistry.

For this purpose, measurements of its sedimentation rate were made according to Svedberg's procedure,² but using the air-driven ultracentrifuge of Bauer and Pickels.³

The two certain criteria of visual purple are its color as determined by its absorption spectrum in the visible region of the spectrum, and its capacity to be bleached by visible light. The absorption spectrum of visual purple in the visible region is an approximately symmetrical band⁴ with a maximum at 500 m μ . While there may be some doubt that all of the absorption on the short-wave side of this maximum is due to visual purple alone and not to contaminating substances, it is certain that the absorption on the long-wave side is due solely to visual purple, since this absorption disappears almost completely after visual purple has been bleached. Therefore, the sedimenting visual purple was photographed with light from the mercury green line 546.1 m μ which falls in the middle of the long-wave side of the absorption spectrum of visual purple.

The light used in photographing the solution did not perceptibly bleach it because the exposures were quite short. However, care had to be taken to shield the sample from extraneous light while preparing it for centrifugation. The filling of the cell, and its location in the rotor were done in a dark room by dim red light; above and below the cell, the openings in the rotor were carefully closed with rubber stoppers, which were removed in the dimly lighted centrifuge room only at the very last moment, just as the rotor was placed in the complete darkness of the vacuum chamber which houses it during operation.

2. The sedimentation velocity of frog's visual purple was measured in each of four samples prepared⁵ as already described and buffered at pH 7.7. In each instance, the speed was 46,800 r. p. m. and the mean centrifugal force 160,000 times gravity. The concentration of each solution was 20 retinas per cc. For convenience the preparations may be referred to as *A*, *B*, *C* and *D*, the order being that in which they were made over a period of five weeks. The photographs and their microphotometer tracings showed only one sedimenting boundary in each experiment. Three of the preparations (*A*, *C*, *D*) were consistent in their behavior while one (*B*) was slightly different. Each solution was stored near 0°C. after preparation and was never more than a day or two old when used for centrifugation. Solutions which are months or years old show differences which are not now understood.

The sedimentation constant, S_{w20} , of a substance as defined by Svedberg² expresses its rate of sedimentation under a set of standard conditions, i.e., in a suspending medium having the density and viscosity of water at 20°C. For its computation from the measured sedimentation rate, there must be known the density and the viscosity of the experimental medium. Determinations of these two quantities were made at different temperatures

on the digitonin-buffer solution used for extracting the visual purple from the retinas. At 20°C., the density was 1.032, and the viscosity 1.058×10^{-2} dyne-sec. per square cm.

The values of the sedimentation constant for preparations *A*, *B*, *C* and *D* turn out to be 11.4, 10.7, 11.2 and 11.1×10^{-13} cm. per dyne per sec., respectively. These give an average value of 11.1×10^{-13} cm. per dyne per sec., from which even the extreme (*B*) deviates by less than 4 per cent. This value of S_{w20} is of the same order of magnitude as those found² for such proteins as edestin, excelsin and phycoerythrin, and leaves no doubt about the fact that visual purple is a relatively large molecule.

3. The absorption spectrum of even the best preparations of visual purple is not quite symmetrical in the visible spectrum; it is distinctly higher on the short-wave side, and the degree of this asymmetry varies with the method of preparation.⁴ With the preparations here used the asymmetry is at a minimum, though it is still present. To determine whether this persistent asymmetry is real or whether it is due to other substances present in the solution, we measured the rate of sedimentation of preparations *C* and *D*, using the mercury blue line 435.8 m μ for photographing the cell. In fact, we so arranged the work that in each experiment these photographs were made alternately with the preceding ones on the same sample that was being centrifuged.

The results show clearly that the asymmetry of the absorption spectrum is an integral property of visual purple because only one boundary was apparent, and the sedimentation constant is the same as that measured with the green line. The respective values of S_{w20} are 11.0, and 11.2×10^{-13} cm. per dyne per sec. and are experimentally indistinguishable from the previous values, the two averages being identical.

4. To determine whether visual purple shows any changes on bleaching, detectable by this method, each of three solutions was bleached at room temperature and kept near a north window for several hours so that all the changes associated with bleaching would be over. Each sample was then centrifuged in the usual way.

Preparation *D* was measured with green and with blue light. The photographs with the green line showed almost no trace of a boundary, thus confirming the notion that the absorption in the green region is due entirely to unbleached molecules. The photographs with the blue line were much like those of the original unbleached solution, and gave a sedimentation constant of 11.3×10^{-13} cm. per dyne per sec. which is experimentally the same as that of the unbleached material. Preparation *A* (bleached) was photographed with ultra-violet light and gave a value of $S_{w20} = 11.2 \times 10^{-13}$. This particular preparation seemed more pure than usual because the measurements of sedimentation rate from photographs taken in the ultra-violet and visible regions are identical. Prepa-

ration *B* again gave slightly aberrant results. The bleached solution measured with blue light yielded an S_{w20} value of 10.3×10^{-13} while with ultra-violet $S_{w20} = 9.7 \times 10^{-13}$. The unbleached solution measured in the ultra-violet gave $S_{w20} = 10.1 \times 10^{-13}$. Very likely, preparation *B* was not as pure as the other three, because the ultra-violet photographs for the unbleached and bleached solutions showed not only the usual boundary but a pronounced density gradient throughout the solution, indicating the presence of some light-absorbing materials having widely different separation rates.

These measurements show that the bleaching of visual purple in solution does not represent any drastic splitting of the molecule; the evidence is compatible with the notion that the bond between the carotenoid portion and the protein portion of the molecule is either rearranged or perhaps broken by light,⁷ but it does not establish either of these ideas.

5. The molecular weight of a protein may be computed from the measured values of its diffusion and sedimentation constants. The diffusion coefficient of visual purple has already been determined¹ in digitonin-buffer solutions at 6°C. The value found under these conditions, $D_6 = 2.2 \times 10^{-7}$ sq. cm./sec., becomes $D_{w20} = 3.5 \times 10^{-7}$ sq. cm./sec. when referred to the standard conditions defining the diffusion constant. However, several days were necessary for carrying out the measurements on which this value is based, and some of the solutions contained a good deal of neutral salt. The value secured may therefore be a little low because visual purple solutions show a tendency toward slow aggregation. This is evidenced by the fact that old solutions, though clear and normally colored, sediment more rapidly than those freshly prepared.

The existence of aggregates is further supported by determinations of the diffusion constant made from the blurred boundaries of the sedimentation photographs. The variations of concentration through the boundaries were not strictly in accord with those attributable to a normal diffusion of single molecules,² indicating some inhomogeneity in the separating particles. There was distinct evidence of some visual purple particles moving slightly faster, in varying degree, than the principal component. The diffusion constant D_{w20} for the principal component secured from the boundaries of sedimentation photographs ranged from 4.5×10^{-7} to 7×10^{-7} sq. cm./sec., the lower values corresponding to measurements taken from the early stages of centrifugation before the inhomogeneities of sedimentation had become accentuated. These lower values are near enough those previously determined to suggest a probably correct value for D_{w20} of 4×10^{-7} sq. cm./sec.

From this diffusion constant and the sedimentation constant, the molecular weight may be computed in terms of Svedberg's well-known equation. The molecular weight of visual purple comes out as 270,000 pro-

vided the molecules have a density equal to that of most proteins, i.e., 1.33 gm./cc. This value of the molecular weight is about one-third of that secured from the diffusion constant alone as previously measured by the method of Anson and Northrop.^{6,1} The difference in the results from the two methods is not unexpected and has been found for other proteins.

The molecular weight may, of course, be computed from the sedimentation constant alone, without use of the diffusion coefficient, provided it is assumed that the molecules are spherical. Applying Stokes's law, the radius of the molecule may be calculated, and if the density is assumed to be 1.33 as before, the molecular weight becomes 200,000. This represents a minimum value, since the spherical form is most favorable for rapid sedimentation. The fair agreement between the two values secured from the sedimentation constant with and without the diffusion constant indicates a shape for the visual purple molecule which is probably not greatly different from that of a slightly oval particle.

* Aided by a grant from the Rockefeller Foundation to Selig Hecht.

¹ S. Hecht, A. M. Chase and S. Shlaer, *Science*, 85, 567 (1937).

² T. Svedberg, *Chem. Reviews*, 20, 81 (1937).

³ J. H. Bauer and E. G. Pickels, *Jour. Exptl. Med.*, 65, 565 (1937).

⁴ E. Koettgen and G. Abelsdorff, *Zeit. Psychol. Physiol. Sinnesorg.*, 12, 161 (1896); G. Wald, *Nature*, 139, 587 (1937); R. J. Lythgoe, *Jour. Physiol.*, 89, 331 (1937); A. M. Chase, *Jour. Gen. Physiol.*, 21 (1938).

⁵ Dr. Aurin M. Chase kindly made these extractions for us.

⁶ M. L. Anson and J. H. Northrop, *Jour. Gen. Physiol.*, 20, 575 (1937).

⁷ G. Wald, *Jour. Gen. Physiol.*, 19, 351 (1935).

BIOLOGICAL DIFFERENCES IN THE ACTION OF SYNTHETIC MALE HORMONES ON THE DIFFERENTIATION OF SEX IN THE CHICK EMBRYO

BY B. H. WILLIER, MARY E. RAWLES AND F. C. KOCH

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF ROCHESTER AND DEPARTMENT OF
BIOCHEMISTRY, UNIVERSITY OF CHICAGO

Communicated March 3, 1938

In a former investigation^{1,2} it was shown that male hormone preparations obtained from male human urine and bull testis act differently upon the developing gonads and gonoducts of the chick embryo. The urinary preparations brought about a feminization of the genetic males just as oestrone and oestriol did. Also they had a masculinizing effect as was shown by (1) the hypertrophy of the potential vasa deferentia (Wolffian ducts) in embryos of both sexes and (2) the inhibition of the oviducts in

genetic females. The bull testis preparations, on the contrary, in the concentrations used produced no changes except an hypertrophy of the Wolffian ducts in both sexes. These biological differences in action were ascribed tentatively to the presence of androsterone and dehydroandrosterone in the urinary preparation and of testosterone in the bull testis preparation.

The purpose of the present paper is to analyze the effects of the synthetic male sex hormones giving particular attention to the biological differences in their action. Either propylene glycol or sesame oil solutions of androsterone, dehydroandrosterone, androstenedione and testosterone propionate were introduced into eggs incubated from 43 to 72 hours, stages either prior to or during the early formation of the gonad primordium. The dosage was single and ranged from 0.02 to 2.4 mgs. Development was then continued until the 16th, 17th or 18th day. The hormones were administered to 300 eggs. Of those treated with androsterone and dehydroandrosterone 34% survived, whereas with testosterone propionate 74% survived, thus indicating a considerable difference in the toxicity of the androgenic substances used. The sex ratio of the surviving embryos is 54 ♀♀ : 64 ♂♂, showing no differential effect. All survivors were examined for changes in the gross anatomy of the gonads and gonoducts. The histology of the gonads and portions of the gonoducts was studied in 76 cases. By using F_1 embryos of the cross Barred Rock ♀ × Rhode Island Red ♂, the original sex was readily ascertained by differences in sex-linked plumage characters.

Genetic Females.—Each of the hormones, except androstenedione which is ineffective in the concentration used (0.09–0.66 mg.), brings about a modification in the form and structure of the gonads and gonoducts of the genetic females (Figs. 2, 4 and 5). Usually with concentrations less than 1.0 mg. the right ovary enlarges by the hypertrophy of the medullary tissue and tends to assume a testis-like shape. The left ovary, on the contrary, remains normal in form and histology. The oviducts are usually typical and the Wolffian ducts are hypertrophied only slightly or not at all. Generally when the dosage exceeds 1.0 mg. both the right ovary and the medulla of the left ovary undergo hypertrophy and the cortex of the left shows changes of a degenerative nature. Both ovaries now assume a testis-like form, the right more so than the left (Fig. 4). The 23 embryos histologically examined may be classified into three grades of effects: (1) Those in which the ovarian cortex of the left gonad is generally thinner than normal, and sterile sex cords (presumably of cortical origin) are adjacent to the medulla. The oviducts are more or less rudimentary and the Wolffian ducts distended with fluid. (2) Embryos in which the ovarian cortex of the left gonad is somewhat degenerate and underlain with sterile sex cords. At the hilus of the gonad the medullary tissue has transformed into solid cords containing germ cells (potential testicular

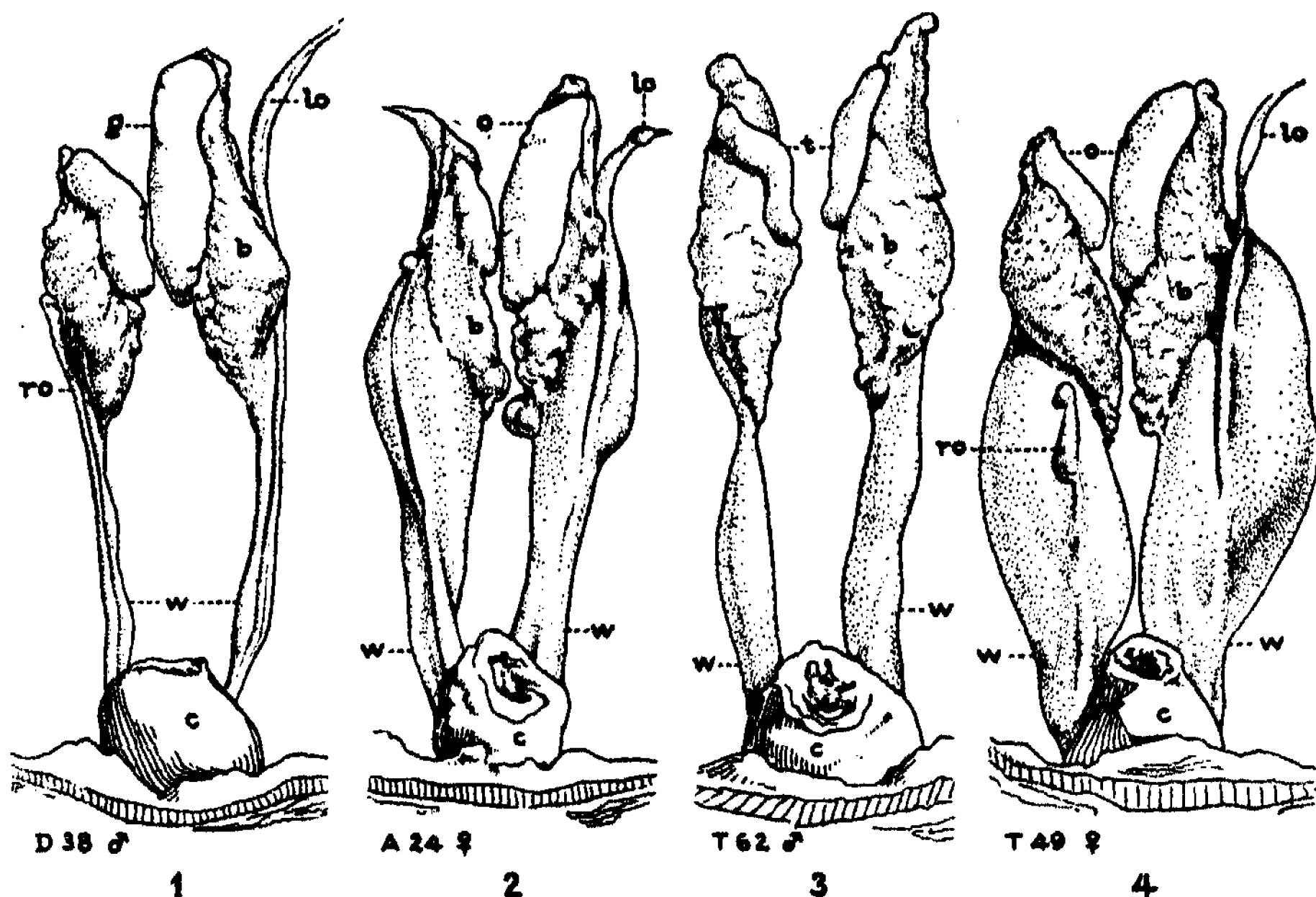


FIGURE 1

Urinogenital system of a 17-day genetic male embryo treated with 1.2 mgs. of dehydroandrosterone. Both right (ro) and left (lo) oviducts well developed; Wolffian ducts (w) moderately swollen; left gonad (g) ovary-like in form, histologically an ovotestis. b, left Wolffian body; c, cloaca. $\times 3.7$.

FIGURE 2

Urinogenital system of a 17-day genetic female given 0.75 mg. of androsterone. Both right and left (o) ovaries have assumed a testis-like form; left oviduct (lo) greatly inhibited—posterior half missing; both Wolffian ducts (w) much distended with fluid. b, right Wolffian body; c, cloaca. $\times 3.7$.

FIGURE 3

Urinogenital system of a 17-day genetic male treated with 1.0 mg. testosterone propionate. Testes (t) small but normal in form. Wolffian bodies (b) and ducts (w) swollen. c, cloaca. $\times 3.7$.

FIGURE 4

Urinogenital system of a 17-day genetic female treated with 2.0 mgs. of testosterone propionate. Ovaries (o) decidedly testicular in form; oviducts, particularly the left (lo), have undergone extreme retrogression; ro, right oviduct; Wolffian bodies (b) and especially the ducts (w) greatly hypertrophied. c, cloaca. $\times 3.7$.

cords). The left oviduct is quite rudimentary, the right either typical or longer than usual. The Wolffian ducts are usually much distended with fluid. (3) Embryos in which cortex of the left gonad is generally more degenerate (even absent over anterior portion) than in the preceding grade. Sterile cords (cortical) are usually present in the outer surface of the medulla and a region of distinct testicular cords with germ cells is seen at the hilus (Fig. 5). Testicular cords likewise appear in the hypertrophied medullary tissue of the right ovary. The left oviduct is generally reduced to a short rudimentary ostial portion containing a lumen, the remainder consisting of oviducal ligaments only; the right, on the contrary, is usually increased in length. The Wolffian ducts and many of the mesonephric tubules become enormously swollen. See figure 4. In general embryos exhibiting this grade of effect occur with a higher frequency when the dosage approaches 2.0 mgs. Finally it should be noted for the embryos of these grades, that the posterior end of the left ovary often exhibits a higher degree of modification in the male direction than the anterior end.

Genetic Males.—The Wolffian ducts and certain mesonephric tubules of the male embryos hypertrophy as they do in the genetic females. The gonads and oviducts, however, respond quite differently to the different hormones. With testosterone propionate the testes are reduced in size but show no essential change in form or structure (Fig. 3). The oviducts never persist. With androsterone and dehydroandrosterone, however, a strong feminizing action is seen which is very similar to that of oesterone and oestriol (Willier, *et al.*²). The left testis changes into a flattened ovary-like body consisting of ovarian and testicular tissues (ovotestis). The right testis remains histologically unchanged until the left testis is strongly feminized whereupon it becomes reduced in size, assumes an ovarian shape and develops some ovarian medullary tissue. The oviducts persist throughout their length and may even hypertrophy. The male thus comes to resemble closely a normal female (Fig. 1).

Degree and Order of Sex Transformation of the Females.—Although a histological study reveals considerable variation in the degree of intersexuality attained in the genetic female embryos for a given dosage, in general it is roughly proportional to the quantity of male hormone administered. The hormones, except testosterone propionate when larger doses are required, are nearly equally effective in producing a given grade of intersexuality. The minimum effective dose of androsterone and dehydroandrosterone is found to be about 0.19 mg. Although this was not ascertained for testosterone propionate a higher dose is indicated since doses between 0.25 and 0.40 mg. were found to be ineffective in nine embryos examined. Furthermore doses of 2.4 mgs. do not bring about any greater effect in the ovary at least than 1.0 mg. of androsterone and de-

hydroandrosterone. The male hormones bring about only a partial sex reversal of the genetic females even though administered in doses as high as 2.0 mgs. and more. This large quantity contrasts strikingly with the action of oestrone and oestriol which in doses of much less than 1.0 mg. may completely feminize a genetic male.

As a rule the first noticeable effect produced in the genetic females is a hypertrophy of the right ovary and of the Wolffian ducts. The left ovary and left oviduct are generally unaffected. Higher doses are necessary to bring about changes in the left ovary and left oviduct. In such cases the ovarian cortex and left oviduct are more or less inhibited and accompanied by structural changes in the ovarian medulla and a distention of both Wolffian ducts with fluid. In low grade modifications the medulla of the left ovary merely hypertrophies but in the higher grades where cortex is more strongly inhibited, the medullary tissue in the hilar region develops into testicular cords (Fig. 5); and many scattered testicular cords likewise develop in the medullary tissue of the right ovary. The formation of testicular cords in the medulla of the left ovary farthest from the ovarian cortex may be indicative of a diminution in the extent of the inhibitory influence which the latter, from ovariectomy studies in the fowl, is known to have upon the ovarian medulla. On theoretical grounds it would be expected that as the ovarian cortex is inhibited more and more by the male hormones the transformation of medulla into testicular cords would spread from the hilus toward the surface. It is interesting to point out that when a testis is converted into an ovary by the action of oestrone or oestriol the order is reversed. That is, the transformation of testicular cords into ovarian medulla proceeds in the direction from the superimposed cortex inward toward the hilus, the cords there retaining their male form last.

Dual Physiological Action.—From the results it is seen that androsterone and dehydroandrosterone have both masculinizing and feminizing effects, whereas testosterone propionate has a masculinizing effect only upon the differentiating gonads and gonoducts. The possibility that testosterone propionate in the concentrations used may have a feminizing effect also upon embryos of other breeds must be recognized since Wolff and Wolff^{2,4} have shown that acetate of testosterone is quantitatively less effective in producing both intersexual males and females of embryos of the Leghorn breed than in F_1 embryos of the cross Light Sussex females \times Rhode Island Red males. Except for a more pronounced hypertrophy on the Wolffian ducts, androsterone is equally as effective as dehydroandrosterone in producing masculinizing and feminizing changes. Testosterone propionate is not greatly different from the other two male sex hormones, except for quantitative differences already noted, in producing masculinizing effects.

The difference in action of androsterone and dehydroandrosterone on the female components of genetic males and females is quite puzzling. As has been noted, these androgens bring about the development of ovarian

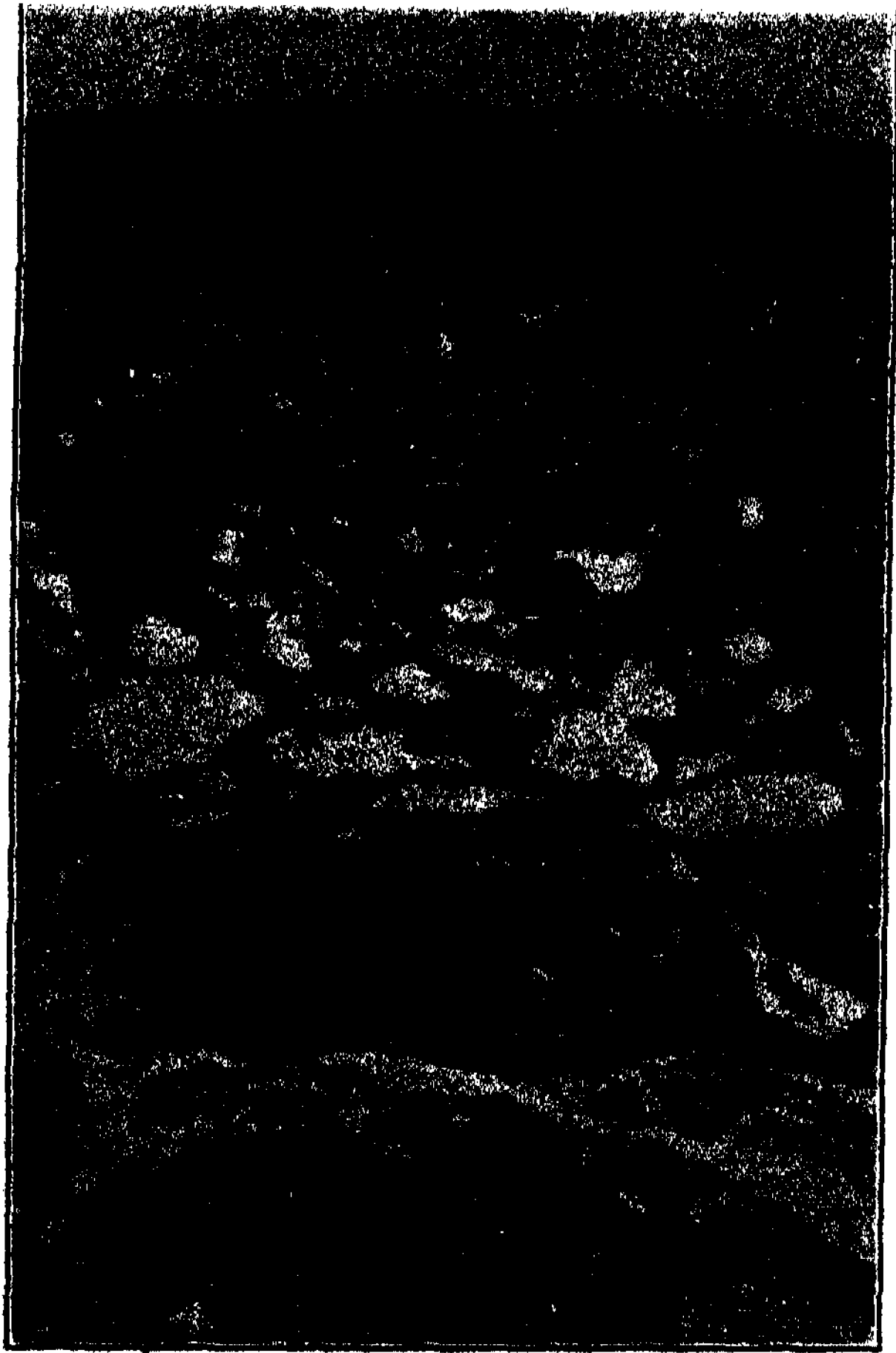


FIGURE 5

Ovotestis of a genetic female treated with 2.0 mgs. of testosterone propionate. Note mass of testicular cords in hilar portion of medulla, i.e., adjacent to adrenal (lowermost tissue in figure). Ovarian cortex thinner than normal. $\times 210$.

cortex and the persistence of the oviducts in genetic males. In genetic females, on the contrary, they have an inhibitory action on these components. That is, the action of these substances in genetic females is wholly masculinizing in nature, whereas in genetic males both feminizing

and masculinizing effects are seen, the former being greater than the latter. The significance of these findings remains for future study.⁵

¹ Willier, B. H., Gallagher, T. F., and Koch, F. C., *Proc. Nat. Acad. Sci.*, **21**, 625-631 (1935).

² Willier, B. H., Gallagher, T. F., and Koch, F. C., *Physiol. Zool.*, **10**, 101-122.

³ Wolff, E., and Wolff, Em., *C. R. Soc. Biol.*, **123**, 1191-1193.

⁴ Wolff, E., and Wolff, Em., *Ibid.*, **124**, 367-369.

⁵ The principal conclusions presented in this paper were communicated to the National Academy of Science in October, 1937 (*Science*, **86**, 409).

THE GENERALIZED CLEBSCH-GORDAN FORMULA

BY F. D. MURNAGHAN

DEPARTMENT OF MATHEMATICS, THE JOHNS HOPKINS UNIVERSITY

Communicated February 23, 1938

The formula of Clebsch-Gordan which furnishes the analysis of the Kronecker product of any two irreducible representations of the two-dimensional unimodular linear group is one of the most important results of group theory in regard to the application of this theory to quantum mechanics; indeed Weyl, in his book on Group Theory and Quantum Mechanics (English edition pp. 128-129) affirms that "it may justly be considered as the fundamental mathematical formula for the classification of atomic spectra and for the theory of the valence bond." We point out here that it is but a special case of a general formula giving the analysis of the Kronecker product of any two irreducible rational, integral representations of the full n -dimensional linear group *which depend on a single label*; the typical rational integral representation $\Gamma_{(\lambda)}$ of the full n -dimensional linear group depends on j labels $(\lambda_1, \dots, \lambda_j)$, $j = 1, 2, \dots, n$ and the generalized Clebsch-Gordan formula has reference to those particular representations for which $j = 1$.

There is also a Clebsch-Gordan formula which furnishes the analysis of the Kronecker product of any two irreducible representations of the 3-dimensional rotation group. We point out here that this is but a special instance of a general formula which furnishes the analysis of the Kronecker product of any two irreducible representations of the full n -dimensional real orthogonal group *which depend on a single label*; the typical irreducible representation $\Gamma_{(\lambda)}$ of the full n -dimensional real orthogonal group depends on j labels $(\lambda_1, \dots, \lambda_j)$, $j = 1, \dots, k$, where $k = \frac{n}{2}$ or $\frac{n-1}{2}$ according as n is even or odd and the generalized Clebsch-Gordan formula has reference to those representations for which $j = 1$. The n -dimensional rotation

group is an invariant subgroup, of dimension 2, of the full real, n -dimensional, orthogonal group so that our generalization is in two directions

(a) from $n = 3$ to n general,

(b) from the rotation group (= proper orthogonal group) to the full real orthogonal group.

The generalization (a), in particular, is not trivial since the whole theory of representations of the orthogonal group is dominated by the number k which for $n = 2$ or 3 is 1 but which > 1 if $n > 3$. We merely state results here; the appropriate demonstrations will be given in a paper to appear shortly in the *American Journal of Mathematics*.

The theorem for the full linear group is well known and is as follows: denoting by $D(\lambda)$ the representation which is specified by the numbers (λ) we have

$$D(\lambda_1) \times D(\lambda_2) = D(\lambda_1, \lambda_2) + D(\lambda_1 + 1, \lambda_2 - 1) + \dots + D(\lambda_1 + \lambda_2).$$

For the two dimensional unimodular group $D(\mu_1, \mu_2) = D(\mu_1 - \mu_2)$, simply because the characteristic numbers of any matrix of this group are each the reciprocal of the other, so that

$$D(\lambda_1) \times D(\lambda_2) = D(\lambda_1 - \lambda_2) + D(\lambda_1 - \lambda_2 + 2) + \dots + D(\lambda_1 + \lambda_2)$$

which is the Clebsch-Gordan formula.

Turning to the full real n -dimensional orthogonal group the generalized Clebsch-Gordan formula is

$$\begin{aligned} D(\lambda_1) \times D(\lambda_2) = & D(\lambda_1 - \lambda_2) + D(\lambda_1 - \lambda_2 + 2) + \dots + D(\lambda_1 + \lambda_2) \\ & + D(\lambda_1 - \lambda_2 + 1, 1) + D(\lambda_1 - \lambda_2 + 3, 1) + D(\lambda_1 + \lambda_2 - 1, 1) \\ & + D(\lambda_1 - \lambda_2 + 2, 2) + \dots \\ & + D(\lambda_1, \lambda_2). \end{aligned}$$

$$\text{E.g., } D(3) \times D(2) = D(5) + D(3) + D(1) + D(4, 1) + D(2, 1) + D(3, 2).$$

When $k = 1$ (i.e., when $n = 2$ or 3) the $D(\mu_1, \mu_2)$ depending on two labels must be modified as follows: when $n = 2$, all $D(\mu_1, \mu_2)$ for which $\mu_2 > 2$ are dropped; all $D(\mu_1, 2)$ are replaced by $-D(\mu_1)$; all $D(\mu_1, 1)$ for which $\mu_1 > 1$ are dropped; and finally $D(1, 1)$ is replaced by $\epsilon D(0)$ where $\epsilon = \pm 1$ according as the element of our group belongs to the rotation subgroup or not. Hence when $n = 2$

$$\begin{aligned} D(\lambda_1) \times D(\lambda_2) &= D(\lambda_1 - \lambda_2) + D(\lambda_1 + \lambda_2); \quad \lambda_1 \neq \lambda_2 \\ D(\lambda_1) \times D(\lambda_1) &= D(0) + D^*(0) + D(2\lambda_1) \end{aligned}$$

the star denoting the associated representation (so that $D^*(0)$ is the alternating one-dimensional representation, $D(0)$ being the identity representation). When $n = 3$, on the other hand, all the $D(\mu_1, \mu_2)$ for which $\mu_2 > 1$ are dropped and all the $D(\mu_1, 1)$ are replaced by $D^*(\mu_1)$ so that

$$\begin{aligned}
 & D(\lambda_1 - \lambda_2) + D(\lambda_1 - \lambda_2 + 2) + \dots + D(\lambda_1 + \lambda_2) \\
 D(\lambda_1) \times D(\lambda_2) = & \\
 & + D^*(\lambda_1 - \lambda_2 + 1) + D^*(\lambda_1 - \lambda_2 + 3) + \dots + D^*(\lambda_1 + \lambda_2 - 1)
 \end{aligned}$$

which is the Clebsch-Gordan formula for the 3-dimensional full real orthogonal. For the rotation subgroup the stars may be removed (since two associated representations of the full group coincide over the rotation subgroup) and we recover the ordinary Clebsch-Gordan formula.

THE ANALYSIS OF REPRESENTATIONS OF THE REAL ORTHOGONAL GROUP

BY F. D. MURNAGHAN

DEPARTMENT OF MATHEMATICS, THE JOHNS HOPKINS UNIVERSITY

Communicated February 23, 1938

Each irreducible rational integral representation $D(\lambda)$ of the full linear n -dimensional group furnishes, by the principle of selection, a representation, in general reducible, of the full real n -dimensional orthogonal subgroup. We indicate here the analysis of this representation of the orthogonal group. On denoting by k the number $\frac{n}{2}$ if n is even and the number $\frac{n-1}{2}$ if n is odd, the analysis of $D(\lambda_1, \dots, \lambda_j)$, $j = 1, 2, \dots, k$, is obtained as follows: let ξ_p , $p = 1, 2, \dots, j$, be an operator which diminishes by unity the label λ_p and let $\Delta(\mu)$ denote the irreducible representation of the full real orthogonal n -dimensional group which is specified by $(\mu) = (\mu_1, \mu_2, \dots)$. Then

$$D(\lambda_1, \dots, \lambda_j) = \left\{ \prod_{p \leq q}^j (1 - \xi_p \xi_q)^{-1} \right\} \Delta(\lambda_1, \dots, \lambda_j).$$

As examples we cite the following. The case $j = 1$ is trivial

$$D(\lambda_1) = (1 - \xi_1^2)^{-1} \Delta(\lambda_1) = \Delta(\lambda_1) + \Delta(\lambda_1 - 2) + \Delta(\lambda_1 - 4) + \dots$$

When $j = 2$ it is convenient to first operate with $(1 - \xi_2^2)^{-1}$ then with $(1 - \xi_1 \xi_2)^{-1}$ and finally with $(1 - \xi_1^2)^{-1}$. In this way we find, for example,

$$D(4, 2) = \Delta(4, 2) + \Delta(4) + \Delta(3, 1) + \Delta(2^2) + 2\Delta(2) + \Delta(0).$$

When $k = 1$ ($n = 2$ or 3) so that $j(= 2) > k(= 1)$ the $\Delta(\mu_1, \mu_2)$ must be modified as follows: when $n = 2$ all $\Delta(\mu_1, \mu_2)$ for which $\mu_2 > 2$ are dropped;

all $\Delta(\mu_1, 2)$ are replaced by $-\Delta(\mu_1)$; all $\Delta(\mu_1, 1)$ for which $\mu_1 > 1$ are dropped; and $\Delta(1, 1)$ must be replaced by $\Delta^*(0)$, the star indicating the associated representation; e.g., when $n = 2$

$$D(4, 2) = \Delta(2) + \Delta(0)$$

(the check by dimensions being $3 = 2 + 1$). If $n = 3$ all $\Delta(\mu_1, \mu_2)$ for which $\mu_2 > 1$ are dropped, and all $\Delta(\mu_1, 1)$ are replaced by $\Delta^*(\mu_1)$. E.g., when $n = 3$,

$$D(4, 2) = \Delta(4) + \Delta^*(3) + 2\Delta(2) + \Delta(0)$$

(the check by dimensions being $27 = 9 + 7 + 10 + 1$).

The modifications just mentioned are only necessary when $j > k$; for a general value of n the necessary modifications are of the same character. When $n = 2k$ is even, every $\Delta(\mu_1, \dots, \mu_k, \mu_{k+1})$ for which $\mu_{k+1} > 2$ is dropped; every $\Delta(\mu_1, \dots, \mu_k, 2)$ is replaced by $-\Delta(\mu_1, \dots, \mu_k)$; every $\Delta(\mu_1, \dots, \mu_k, 1)$ for which $\mu_k > 1$ is dropped and every $\Delta(\mu_1, \dots, \mu_{k-1}, 1^2)$ is replaced by $\Delta^*(\mu_1, \dots, \mu_{k-1})$. When $n = 2k + 1$ is odd, every $\Delta(\mu_1, \dots, \mu_k, \mu_{k+1})$ for which $\mu_{k+1} > 1$ is dropped while each $\Delta(\mu_1, \dots, \mu_k, 1)$ is replaced by $\Delta^*(\mu_1, \dots, \mu_k)$. Similar, but of necessity more elaborate, "modification rules" may be given when $j > k + 1$ (the maximum value of j being n).

We close with the following remarks: The analysis of the representations of the full real orthogonal group which are obtained by the principle of selection from the irreducible rational integral representations of the full linear group furnishes the analysis of the representations of the rotation (= proper real orthogonal) group which are similarly obtained. The method here described is also available (and simpler in application) for the complex group.

NATURAL SYSTEMS

BY ALFRED L. FOSTER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF CALIFORNIA

Communicated February 21, 1938

1. A system with single composition (N, \circ) , is a *natural system*, if it can be faithfully realized by some non-empty set of natural numbers ≥ 2 which is closed with respect to (and where \circ is realized as) ordinary multiplication. In connection with natural systems, the definitions of such terms as are borrowed from the ordinary natural number system will be clear by analogy, and need not be explicitly given in this summary. There

are then exactly N_0 (abstractly) different natural systems N_1, N_2, \dots, N_{N_0} , where N_k is that system having exactly k prime elements.

A *regular ordering* of N is an infinite sequence consisting of the elements of N , each exactly once, and such that $\xi < \eta$ implies $\rho\xi < \rho\eta$, for all elements ξ, η, ρ of N , where $<$ is an abbreviation for *precedes*, and $\rho\xi$ for $\rho \circ \xi$.

THEOREM 1. *In a regular ordering of (N, \circ) each element is preceded by each of its proper divisors; in particular the first element must be a prime.*

Two regular orderings of (N, \circ) are *essentially different* if they cannot be made identical by any relettering (permutation) of their primes.

As an important step in the study of the structure of the regular orderings of (N, \circ) , a problem which bears heavily on both the theory of integers and of real numbers, this summary deals with principal concepts and results on the simplest non-trivial case, the natural system N_2 . The main theorems are 4, 6, 9.

2. Let α, β denote the prime elements of and E the class of all essentially different regular orderings of M_2 , i.e., the class of all orderings of N_2 for which $\alpha < \beta$. An *inequality* is a relation either of the form $\alpha^m < \beta^{m'}$, or else $\beta^{n'} < \alpha^n$. (Here $\alpha^m = \alpha \cdot \alpha \cdot \dots \cdot \alpha$, m factors; $m = 1, 2, \dots$). Each element of E is uniquely determined by a suitable (necessarily infinite) list of inequalities. Again, certain subclasses of E are definable by a finite list of inequalities. Such subsets of E we call *rational*. Lists of inequalities which define the same subset of E are *equivalent*.

A *reduced list* is one consisting either of a single inequality of type (1°): $(\alpha^m < \beta^{m'})$, or else of a pair of inequalities of type (2°): $\begin{pmatrix} \alpha^m < \beta^{m'} \\ \beta^{n'} < \alpha^n \end{pmatrix}$, where in each case we demand $(m, m') = 1$ (i.e., relatively prime), $m' \leq m$, $(n, n') = 1$, $mn' < m'n$.

THEOREM 2. *Each rational subclass of E may be defined by exactly one reduced list. Conversely, each reduced list defines exactly one rational subclass of E .*

A *primitive list* is one consisting either of a single inequality of form (i): $(\alpha^m < \beta)$, or else of a pair of inequalities of form (ii): $\begin{pmatrix} \alpha^m < \beta^{m'} \\ \beta^{n'} < \alpha^n \end{pmatrix}$, with $m' \leq m$, and $mn' + 1 = m'n$. A primitive list is obviously reduced. A (*rational*) *primitive subclass* (of type (i) or (ii), respectively) of E is one whose reduced defining list is primitive (of type (i) or (ii), respectively). With the exception of the last theorem, 9, primitive class is used to mean rational primitive class. In theorem 9, however, for symmetry of expression, we call a single element of E an *irrational primitive class*.

3. The pair $[\alpha', \beta']$ is the *first undetermined pair* of a rational subclass R of E if r is the smallest integer for which an x exists such that $\alpha^r < \beta^x$ for at least one, and also $\beta^{x'} < \alpha^r$ for at least one element of R , and r' is the smallest x satisfying the above. The integers r, r' of $[\alpha', \beta']$

clearly satisfy: $(r, r') = 1$, and $r > r'$. The rational number $r/r' > 1$ we call the *characteristic number* of the rational subclass R .

THEOREM 3. *The characteristic number of a primitive class P is the rational integer $m + 1$ if P is of type (i), and the rational non-integer $\frac{m + n}{m' + n'}$ if P is of type (ii).*

By use of this theorem one proves the basic

THEOREM 4. *Each rational number > 1 is the characteristic number of one and only one primitive subclass of E . Moreover, to a rational integer (respectively non-integer) corresponds a primitive subset of type (i) (respectively (ii)).*

Accordingly we uniquely denote by E_v that primitive subclass of E whose characteristic number is the rational number $v > 1$, and in this way exhaust the primitive subclasses.

4. As examples of numerous decomposition theorems we mention two.

THEOREM 5. *If the logical product of two primitive subclasses of E is not empty, then one of the primitive subclasses is a subclass of the other.*

THEOREM 6. *Every rational subclass of E can be expressed as the logical sum of a finite number of disjoint primitive classes the logical sum of no two of which is again a primitive subclass. Moreover, this decomposition is unique.*

5. Any single element J of E may be defined by a denumerably infinite list of pairs of inequalities, such that each pair defines a primitive class E_{v_1} , and such that in the sequence E_{v_1}, E_{v_2}, \dots each $E_{v_{n+1}}$ is a proper subset of E_{v_n} where v_n is the characteristic number of the principal class E_{v_n} . Such a sequence of primitive subclasses we call *regular*. By use of decomposition theorems one shows

THEOREM 7. $\lim_{n \rightarrow \infty} v_n$ exists, and is independent of the regular sequence of principal classes which is used to define the element J of E .

We then call $\lim_{n \rightarrow \infty} v_n$ the *characteristic number* of the element J of E . From theorem 4 we then get

THEOREM 8. *The characteristic number of a single element of E is an irrational number.*

Then by another appeal to decomposition theorems one extends theorem 4 to

THEOREM 9. FUNDAMENTAL THEOREM. *To each primitive subclass* of E corresponds a unique real number > 1 , the characteristic number of the subclass. Conversely, each real number > 1 is the characteristic number of a unique primitive subset of E . Moreover, to an irrational number corresponds an irrational primitive subclass, while to a rational integer (non-integer) corresponds a rational primitive subset of type (i) (type (ii)).*

* See closing remarks of §2.

EXTENSIONS OF MEASURE

BY H. M. MACNEILLE

DEPARTMENT OF MATHEMATICS, HARVARD UNIVERSITY

Communicated March 14, 1938

Introduction.—One of the elementary applications of measure theory is, using two dimensional terminology, the determination of areas in the Euclidean plane. Initially, area is defined only for certain rectangular or elementary figures. It is the object of the theory of measure to extend the definition of area to as large a class of subsets of the plane as possible. The set of figures which can be formed by a finite number of rectangles with sides parallel to fixed directions and with vertices with rational coördinates is dense and denumerable. This set plays a rôle in the theory of measure analogous to that of the rational numbers in the construction of the real numbers. It is the object of this note to give an abstract formulation for measure theoretical constructions analogous to the various methods of defining real numbers in terms of rationals.¹

One starts with an abstract system of point sets which has the pertinent properties of the set of rectangular figures in the plane. This set is extended by methods analogous to those by which the real numbers are obtained. The elements of the extended sets so obtained are abstract, that is, they are not point sets, as were the elements of the original set. The next step is the assignment of point sets to these abstract elements. Certain of these point sets may be ordinate sets. To these, functions can be assigned and, by this means, integration² is introduced. The integral of a non-negative function is the measure, or area, of its ordinate set. In particular, the ordinate set of a step function is a rectangular figure.

A real number may be defined as a sequence of nested intervals of rationals. The analogue of this method yields the theory of content and Riemann integration. The analogue of the definition of real numbers by fundamental, or Cauchy, sequences gives Lebesgue measure and integration. The method of cuts does not lend itself readily to interpretation in terms of measure. The method of bounded monotone sequences, when applied first with ascending and then with descending sequences, is, in essence, the classical method for the definition of Lebesgue measure. A precise formulation of the first two of these methods follows.

Definitions and Notation.—The domain of discussion is a basic set, I , of points. Subsets of I are denoted by a, b, c, \dots . The symmetric difference, $a - b$, is the set of points in either a or b but not in both. $a + b$ is the set of points in either a or b or in both. The iteration of this operation either a finite or an infinite number of times is denoted by Σ . ab is the set of points in both a and b and iteration of this operation is denoted

by II. If every point of a is a point of b , then $a \subset b$. If a and b contain the same points, then $a = b$. The identity $a + b = a - b - ab$ and the equivalence of $a \subset b$ and $a = ab$ may be used to define the operation $+$ and the relation \subset .

A Boolean ring³ is an algebraic ring in which $aa = a$ for every element in the ring. Multiplication in such a ring is necessarily commutative and addition is modulo 2. Consequently, addition and subtraction are the same and the minus sign can be used throughout to denote this operation. By this convention ring addition can be associated with point set symmetric difference and the plus sign reserved for point set addition. A system of point sets is a Boolean ring if point set symmetric difference is identified with ring addition, point set multiplication, with ring multiplication and point set equality, with ring equality, provided that the system is closed under these operations. In general, not all the subsets of the basic set will be elements of a given Boolean ring of point sets. In fact, the void subset, 0, is the only subset which is necessarily an element of such a ring. The letter K will be used to denote a Boolean ring of subsets of I for the elements of which a real valued function, the absolute value, satisfying the following conditions is defined.

1. $|a| > 0$, if $a \neq 0$.
2. If $a = b$, then $|a| = |b|$.
3. $|a| = |a - ab| + |ab|$.

As consequences of these conditions, it follows that:

- (a) If $a \subset b$, then $|a| \leq |b|$;
- (b) $|a + b| = |a| + |b| - |ab| \leq |a| + |b|$;
- (c) $|a - b| = |a| + |b| - 2|ab| \leq |a| + |b|$.

The absolute value in K is said to be completely additive if, in addition to Conditions 1, 2 and 3, the following condition holds.

4. If $a = \sum_{i=1}^{\infty} a_i$ and $a_i a_j = 0$ for $i \neq j$, then $|a| = \sum_{i=1}^{\infty} |a_i|$.

Conditions equivalent to 4 follow:

- 4'. If $a = \sum_{i=1}^{\infty} a_i$, then $|a| = \lim_{n \rightarrow \infty} \left| \sum_{i=1}^n a_i \right|$;

4". If $\{a_i\}$ is a sequence such that $a_i \subset a_j$ for $i \geq j$ and $\lim_{i \rightarrow \infty} |a_i| = k > 0$, then there is a point common to every a_i .

Condition 4 is a covering property similar to the Heine-Borel theorem for open sets. Condition 4" is analogous to the Cantor property of descending sequences of closed sets. In fact, measurable sets may be re-

garded as both open and closed in a suitably defined discrete space. Nothing in Conditions 4, 4' and 4'' implies the existence of infinite sums or products in K .

Any system of elements for every pair of which a real valued function, $\rho(a, b)$, is defined such that $\rho(a, a) = 0$, $\rho(c, a) \leq \rho(a, b) + \rho(b, c)$ and in which equality is defined by the relation $a = b$ if $\rho(a, b) = 0$ is a metric space. If, by definition, $\rho(a, b) = |a - b|$, then K is a metric space.

Examples.—For an example of a system K , let I be n dimensional Euclidean space, E_n , of points (x_i) , $i = 1, 2, \dots, n$, where the x_i are real numbers. A half open interval in E_n is determined by two points (a_i) and (b_i) such that $a_i < b_i$ for all i , and consists of all points (x_i) such that $a_i \leq x_i < b_i$ for all i . The absolute value of an interval is defined as $\prod_{i=1}^n (b_i - a_i)$.

If, for all i , a_i and b_i are rational, then the interval is said to be rational. Let $K(E_n)$ be the system whose elements are the sets in E_n which can be represented as the sum of a finite number of disjoint rational half open intervals. The absolute value of an element in $K(E_n)$ is the sum of the absolute values of the intervals by which it is represented. This definition leads to no ambiguities. $K(E_n)$ satisfies all the conditions for K and the absolute value is completely additive.

Let K and K' be Boolean rings of subsets of I and I' for which absolute values have been defined. Let $I'' = I \times I'$, the set of pairs of points, the first, from I , the second, from I' . If $a \in K$ and $a' \in K'$, let (a, a') represent the points in I'' formed by a point from a and a point from a' . Let $|(a, a')| = |a| |a'|$. Denote the set of all such elements by $K \times K'$. Let a subset of I'' be an element of K'' if, and only if, it can be represented as the sum of a finite number of disjoint elements in $K \times K'$. The absolute value of an element in K'' is defined as the sum of the absolute values of the elements of $K \times K'$ by which it is represented. This definition⁴ leads to no ambiguities. K'' fulfils all the conditions imposed on K and K' . In particular, if the absolute values in K and K' are completely additive, then so is the absolute value in K'' . If K and K' are taken as $K(E_n)$ and $K(E_m)$, respectively, then K'' is $K(E_{n+m})$.

Extension by Sequences of Nested Intervals.—The extension of a system, K , by means of sequences of nested intervals, is now considered. The extended set will be denoted by L .

DEFINITION. $[a_n, b_n]$, $n = 1, 2, \dots$, is a sequence of nested intervals in K if $a_n \subset a_m \subset b_m \subset b_n$, for $n \leq m$.

DEFINITION. The extended system, L , is defined as follows:

(a) The elements of L are the sequences of nested intervals in K for which $\lim_{n \rightarrow \infty} |a_n - b_n| = 0$;

(b) $[a_n, b_n] = [c_n, d_n]$, if $a_n \subset d_n$ and $c_n \subset b_n$;

- (c) $[a_n, b_n] - [c_n, d_n] = [(a_n - c_n)(b_n - d_n), (b_n + d_n) - a_n c_n];$
 (d) $[a_n, b_n][c_n, d_n] = [a_n c_n, b_n d_n];$
 (e) $|[a_n, b_n]| = \lim_{n \rightarrow \infty} |a_n|.$

THEOREM. L is a Boolean ring with absolute value. The correspondence $a \longleftrightarrow [a_n, a_n]$, where $a_n = a$ for every n , is a one to one mapping of K onto a subset of L . The algebraic operations, absolute value and equality are preserved by this mapping. L is closed to further extension by this method.

The elements of L are not point sets. The next step is the assignment of subsets of I to the elements of L . The point set operations of the assigned sets must correspond to the algebraic operations in L of the elements to which the sets belong. The absolute value of an element in L will be the content of the point sets which belong to it.

DEFINITION. A subset, c , of I belongs to an element $[a_n, b_n]$ of L if $\sum_{n=1}^{\infty} a_n \subset c \subset \prod_{n=1}^{\infty} b_n$. If c belongs to $[a_n, b_n]$, then $|[a_n, b_n]|$ is the content of c .

Every element of L has at least one point set, $\sum_{n=1}^{\infty} a_n$, belonging to it.

In general, not every subset of I will belong to an element of L . Let G be the class of subsets of I which belong to some element of L . Let Y be the class of point sets with content zero.

THEOREM. G is a Boolean ring of point sets. Y is an ideal in G . The quotient ring G/Y is isomorphic with L . K is a subring of G and the absolute value of an element in K is equal to its content.

When the given Boolean ring is $K(E_n)$, G is the class of sets in E_n for which Jordan content is defined. An effective extension is secured in this case. The Riemann integral of a function is the content of its ordinate set. The theory of Riemann integration can be developed from this point, the general procedure being to establish properties in $K(E_n)$, that is, for step functions, and then showing that the properties are preserved under the extension.

For the extension from K to G it is not necessary that the absolute value in K be completely additive. However, the complete additivity of the absolute value in K leads to the following theorem.

THEOREM. If the absolute value in K is completely additive, then so is it in G . Furthermore, if $[a_n, b_n]$ is any sequence of nested intervals and

$$\sum_{n=1}^{\infty} a_n = \prod_{n=1}^{\infty} b_n, \text{ then } \lim_{n \rightarrow \infty} |a_n - b_n| = 0.$$

Extension by Fundamental Sequences.—The extension, by fundamental sequences, of a system K with completely additive absolute value is considered next.

DEFINITION. A sequence, $[a_n]$, in K is a *fundamental sequence* if, for

every $\epsilon > 0$, an integer, $N(\epsilon)$, exists such that whenever $m > n > N(\epsilon)$ $|a_n - a_m| < \epsilon$.

DEFINITION. A sequence, $[a_n]$, in K is *absolutely convergent* if $\sum_{n=1}^{\infty} |a_n - a_{n+1}|$ is convergent.

THEOREM. In K , every absolutely convergent sequence is a fundamental sequence and every fundamental sequence contains an absolutely convergent subsequence.

No generality is lost by restricting the argument to absolutely convergent sequences instead of admitting the entire class of fundamental sequences.

DEFINITION. The extended set, M , is defined as follows:

- (a) The elements of M are the absolutely convergent sequences in K ;
- (b) $[a_n] = [b_n]$, if $\lim_{n \rightarrow \infty} |a_n - b_n| = 0$;
- (c) $[a_n] - [b_n] = [a_n - b_n]$;
- (d) $[a_n][b_n] = [a_n b_n]$;
- (e) $|[a_n]| = \lim_{n \rightarrow \infty} |a_n|$.

If K is regarded as a metric space, then M is the completion of K by the Cantor-Meray⁵ process. However, in addition to this, the algebraic operations of K have also been extended to M . The extension from K to M , here defined by sequences, can equally well be formulated in terms of infinite series, that is, infinite symmetric differences.

THEOREM. M is a Boolean ring with absolute value. Every fundamental sequence in M has a limit in M . The correspondence, $a \longleftrightarrow [a_n]$, where $a_n = a$ for all n , is a one to one map of K onto a subset of M which preserves the algebraic operations, absolute value and equality. M is closed to further extension by this method.

The complete additivity of the absolute value in K is not necessary for the construction of M , but it is necessary if the measure of the point sets assigned to elements of M is to be uniquely defined.

DEFINITION. A subset, a , of I belongs to an element, $[a_n]$, in M if $\prod_{n=1}^{\infty} \sum_{i=n}^{\infty} a_i \supset a \supset \sum_{n=1}^{\infty} \prod_{i=n}^{\infty} a_i$. If a belongs to $[a_n]$, then $|[a_n]|$ is the *measure* of a .

Under this definition, if a belongs to $[a_n]$, then a contains every point which occurs in all but a finite number of terms of $[a_n]$ and all points which occur in but a finite number of terms, or not at all, are excluded from a . The remaining points may or may not be in a . Their measure is zero.

At least one subset of I belongs to each element of L , namely $\prod_{n=1}^{\infty} \sum_{i=n}^{\infty} a_i$. In general, not every subset of I belongs to an element of M . Let H be the

class of subsets of I which belong to elements of M . Let Z be the class of sets of measure zero.

THEOREM. *If the absolute value in K is completely additive, then so is the measure in H . If the void set belongs to $[a_n]$, then $||[a_n]|| = 0$.*

THEOREM. *If a belongs to $[a_n]$ and b belongs to $[b_n]$, then ab belongs to $[a_nb_n + (a_n - a_{n+1})(b_n - b_{n+1})] = [a_nb_n]$ and $a - b$ belongs to*

$$[a_n - b_n - b_n(a_n - a_{n+1})(b_n - b_{n+1})] = [a_n - b_n].$$

THEOREM. *H is a Boolean ring of point sets. Z is an ideal in H . The quotient ring H/Z is isomorphic with M . K and G are subrings of H and, in G , the content of a set is equal to its measure.*

When the given system is $K(E_n)$, H is the class of subsets of E_n for which Lebesgue measure is defined and finite, and the measure in H is Lebesgue measure. The theory of Lebesgue integration can be developed from this point by assigning functions to Lebesgue measurable ordinate sets.

¹ The possibility of such constructions has been remarked by Gillis, J., *Quart. Jour. Math.*, 7, 192, §2 (1936) and Glivenko, V., *Am. Jour. Math.*, 58, 802, §7 (1936).

² In this paper, integration has been made subordinate to measure. Analogous constructions in which integration is fundamental are possible. Measure is introduced by means of characteristic functions. Such a construction, by means of monotone sequences, is given by Daniel, P. J., *Ann. Math.*, 19, 279-294 (1917-18) and 21, 203-220 (1919-20). The method of fundamental sequences has been used to define an integral by Dunford, N., *Trans. Am. Math. Soc.*, 37, 441-453 and 38, 600-601 (1935).

Constructions of this type by M. H. Stone and N. Wiener have not been published.

³ For formal algebraic properties of Boolean rings, see Stone, M. H., *Trans. Am. Math. Soc.*, 40, 37-111, Chapter I (1936).

⁴ For the definition of measure in product spaces, see Lomnicki, Z., and Ulam, S., *Fund. Math.*, 23, 237-278 (1934).

⁵ Hausdorff, F., *Mengenlehre*, Leipzig, Gruyter, (2nd. Ed., 1927), p. 106.

REPRESENTATION AND EQUIVALENCE OF FORMS

BY RUFUS OLDENBURGER

• INSTITUTE FOR ADVANCED STUDY

Communicated February 26, 1938

This paper is concerned with the expressibility of a form F with coefficients in a given field as a sum S of powers of linear forms, and the determination of various possible representations S of F . These representations are employed to give necessary and sufficient conditions for the equivalence of forms of any degree under non-singular linear transformations in a given field. Until the present paper no such conditions were obtained for the classical problem of equivalence of general forms of degree higher than 2. It was for the purpose of solving this general equivalence problem that the

author developed the theory of higher dimensional matrices in earlier papers.

1. *Existence of Representations.*—The matrix $A = (a_{ij} \dots_m)$ associated with a form $F = a_{ij} \dots_m x_i x_j \dots x_m [i, j, \dots, m = 1, 2, \dots, n]$ is said to be *symmetric* if the value of an element of A is invariant under permutation of its subscripts. Let S denote the sum

$$S = \lambda_1 L_1^p + \dots + \lambda_\sigma L_\sigma^p, \quad (1.1)$$

where $\lambda_1, \dots, \lambda_\sigma$ are coefficients, and L_1, \dots, L_σ are linear forms. We shall say that a form F can be written in a field ϕ as a *sum of pth powers of linear forms* if there exists an S such that $F = S$, where $\lambda_1, \dots, \lambda_\sigma$ and the coefficients of L_1, \dots, L_σ are in ϕ . Our basic theorem on the existence of representations of F is the following.

THEOREM 1.1. *Let $[F]$ denote the class of forms of degree p with symmetric matrices whose coefficients are in a field ϕ . Let $[F_S]$ denote the sub-class of forms of $[F]$ which can be written in ϕ as sums of pth powers of linear forms. If ϕ is composed of p or more elements the classes $[F]$ and $[F_S]$ are identical.*

Writing $L_i = a_{ij}x_j$ and F as above, the equation $F = S$ is satisfied if the system of equations

$$\sum_{\alpha=1}^{\sigma} \lambda_{\alpha} a_{\alpha i} a_{\alpha j} \dots a_{\alpha m} = a_{ij} \dots_m, \quad (1.2)$$

which are linear in the λ 's is satisfied. There are as many equations in (1.2) as distinct elements N in the general symmetric matrix. Let $\sigma = N$. By restricting the elements of $(a_{\alpha i})$ in a very special way we can write the matrix M of coefficients of the λ 's in (1.2) as a matrix M' with diagonal minors of the form

$$K = (\alpha_i^r \alpha_j^s \dots \alpha_m^t),$$

where (r, s, \dots, t) and (i, j, \dots, m) form multipartite row and column indices of K , respectively, and i, \dots, m, r, \dots, t are indices ranging over sets of values subject to the restrictions

$$\begin{aligned} i + j + \dots + m &\leq p - 1, \\ i, j, \dots, m &\geq 0, \\ r + s + \dots + t &\leq p - 1, \\ r, s, \dots, t &\geq 0. \end{aligned}$$

The non-diagonal minors of M' are zero. By an induction process in which K is reduced under elementary transformations to a matrix whose diagonal minors are of the same type as K we prove that the determinant $|M|$ is non-singular if $\alpha_0 = 1$, and $\alpha_0, \dots, \alpha_{p-1}$ are distinct.

That the classes $[F]$ and $[F_S]$ are not always identical follows from the fact that x^2y cannot be written as a sum S for the field composed of the

elements 0, 1 with characteristic 2. Let $[F]_{np}$ denote the class of forms of degree p in n variables with coefficients in ϕ . Let $[F_S]_{np}$ denote the subclass of $[F]_{np}$ of forms F which have a representation (1.1) in ϕ . That there exist values of n and p such that $[F]_{np}$ and $[F_S]_{np}$ are identical for every field ϕ follows from the fact that these classes are equal for binary forms of the fourth degree and any field.

2. *Properties of Representations.*—If in a field ϕ we can write a form F as (1.1) we shall say that F has a representation $[\lambda_\alpha, A]$ in ϕ where λ_α denotes the set $\lambda_1, \dots, \lambda_\sigma$, and A is the matrix $(a_{\alpha i})$ of coefficients of the $L_\alpha = a_{\alpha i}x_i$ in (1.1). A representation $[\lambda_\alpha, A]$ of F for which σ takes on its smallest value is said to be a *minimal representation* of F in ϕ . Let a field for which the symmetric matrix of a form F of degree p in n variables is unique be termed an (n, p) -*proper field*. From the rank of the matrix M of coefficients of the λ 's in equations (1.2), and the non-vanishing of the λ 's we prove the theorem which follows.

THEOREM 2.1. *Let a form F be of degree p in n variables. In a minimal representation $[\lambda_\alpha, A]$ of F for an (n, p) -proper field the λ 's are uniquely determined by A .*

We shall understand that "equivalence" of forms means equivalence under non-singular linear transformations on the variables occurring in the forms. Since m by n matrices A and A' where $m \geq n$ are related by an equation $A' = UA$, where U is non-singular, and further a minimal representation $[\lambda_\alpha, A]$ transforms into a minimal representation $[\lambda_\alpha, A']$ under non-singular linear transformations we derive the following equivalence theorem.

THEOREM 2.2. *Let $[\lambda_\alpha, A]$ be a minimal representation of a form F in ϕ . For the equivalence of a form F' to F in ϕ it is necessary that F' have a representation $[\lambda_\alpha, A']$ for some A' with respect to ϕ . If this necessary condition is satisfied the forms F and F' are equivalent in ϕ if and only there exist non-singular matrices U, X with elements in ϕ such that*

$$A' = UAX,$$

where $[\lambda_\alpha, UA]$ is a representation of F .

3. *Non-Singular Representations.*—If $[\lambda_\alpha, A]$ is a minimal representation of F in a field ϕ , then $[\lambda'_\alpha, DJA]$ is a minimal representation of F in ϕ , where the λ'_α are equal in some order to the λ_α multiplied by p th powers of non-zero elements in ϕ , J is a permutation matrix, and D is a diagonal matrix depending on the λ'_α . If A is non-singular the only other minimal representations of a form F of degree p in n variables for an (n, p) -proper field ϕ are of this form. Thus theorem 2.1 can be strengthened in this case to the following theorem.

THEOREM 3.1. *Let F be a form of degree p in n variables with a representation $[\lambda_\alpha, A]$ in an (n, p) -proper field ϕ , where A is non-singular. The*

matrix A determines the λ 's uniquely, and the λ 's determine A up to a factor DJ on the left.

The form F is equivalent in ϕ to a form F' with diagonal matrix if and only if F has a minimal representation $[\lambda_\alpha, A]$ where A is non-singular. Then $F' = \lambda_1 x_1^p + \dots + \lambda_n x_n^p$.

4. *Associated Multilinear Forms.*—With a form $F = a_{ij} \dots_m x_i x_j \dots x_m$ having a symmetric matrix there is associated a unique multilinear form M_F given by $a_{ij} \dots_m x_i y_j \dots z_m$ which can be obtained from F by the well-known Aronhold polarization process provided that the field of operations is (n, p) -proper if F is of degree p in n variables. Concerning multilinear forms we have the following theorem.

THEOREM 4.1. *A multilinear form $M = b_{ij} \dots_m x_i y_j \dots z_m$ can be written in a given field as*

$$\sum_{\alpha=1}^{\sigma} L_{\alpha} M_{\alpha} \dots N_{\alpha}, \quad (4.1)$$

where the L_{α} are linear forms in the x 's only, the M_{α} are linear forms in the y 's only, \dots , and finally the N_{α} are linear in the z 's only.

If $1, 2, \dots, n$ is the maximum range of the indices i, j, \dots, m the form M has a representation (4.1) with $\sigma \leq n^{p-1}$ where p is the number of vectors in the set $(x_i), \dots, (z_m)$.

The minimum value of σ for which a form M_F can be written as (4.1) in a field ϕ is called the *factorization rank*¹ of F in ϕ . The minimum value of σ for which F can be written as (1.1) is termed the *minimal number* of F . Let $[F]$ now denote the class of forms F defined by the properties

1. The coefficients of F are in a field ϕ , and $(a_i \dots_m)$ is symmetric.
2. F is of degree p .
3. F involves n variables and cannot be reduced by means of non-singular linear transformations in ϕ to a form with less than n variables.

The minimum value of the factorization ranks of forms in $[F]$ with respect to ϕ is n ; this is also true of minimal numbers. Making use of symmetry properties of the matrix $(a_{ij} \dots_m)$ of F and rank properties of direct products of 2-way matrices we prove the following theorem which is valid for an (n, p) -proper field.

THEOREM 4.2. *If the minimal number of a form F in $[F]$ is a minimum for $[F]$, the factorization rank of F is a minimum for $[F]$, and these minimum values are equal; and conversely.*

If the minimum value of the minimal number is taken on for F , the form F is equivalent to a form with diagonal matrix, and M_F is non-singular in the sense of another paper² of the author. Conditions for non-singularity of M_F were given in that paper which may hence be applied to F to determine whether or not F is equivalent to a form with diagonal matrix. By

properties of generalized determinants proved elsewhere³ we have the following theorem.

THEOREM 4.3. *For an (n, p) -proper field ϕ a form F in $[F]$ of odd degree is equivalent to a form with diagonal matrix if and only if the generalized determinant⁴ $D = |x_i a_{ij} \dots|$ signant on j, \dots, m factors in ϕ into linearly independent linear factors and under reduction in ϕ of D to $kx_1 \dots x_n$ by non-singular linear transformations, F transforms covariantly to a form with diagonal matrix.*

A modification of this theorem holds for forms of even degree. For the complex field the factorability of D into linearly independent linear factors may be recognized with the aid of conditions obtained by Hocevar⁵ for the reducibility of a form into linear factors.

We write $F = x_i F_i$, where F is a form in x_1, \dots, x_n . We term F_1, \dots, F_n the sub-forms of F . By rank properties of the associated p -way matrix of F we prove the following theorem.

THEOREM 4.4. *For an (n, p) -proper field ϕ a form F of $[F]$ of degree $p \geq 3$ is equivalent to a form with diagonal matrix if and only if the sub-forms of F are equivalent in ϕ to forms with diagonal matrices.*

If F is of degree ≥ 3 , and has a representation $[\lambda_\alpha, A]$ where A is non-singular there exist numbers ρ_i in the given field such that $\rho_i F_i$ has a representation $[\lambda'_\alpha, A']$ where A' is non-singular. By this property, theorem 4.4, and the fact that the transformations which bring a form of degree ≥ 3 with diagonal matrix into a form with diagonal matrix bring any form with diagonal matrix into a like form we prove the following theorem.

THEOREM 4.5. *Let $F = x_i F_i$. For an (n, p) -proper field ϕ a form F of the class $[F]$ is equivalent to a form with diagonal matrix if and only if the following conditions are satisfied.*

a. *There exist numbers ρ_i in ϕ such that $\rho_1 F_1 + \dots + \rho_n F_n$ has a representation $[\lambda_\alpha, A]$ in ϕ where A is non-singular, and $\lambda_\alpha \neq 0$ for each α .*

b. *If condition a is satisfied, assume that $\rho_1 \neq 0$ and reduce $\rho_1 F_1$ by a non-singular linear transformation T in ϕ to a form F' with diagonal matrix. Let the transformation T bring F_2, \dots, F_n into F'_2, \dots, F'_n . Then F'_2, \dots, F'_n are forms with diagonal matrices.*

5. *Ranks and the Minimal Number.*—The values of the factorization rank and minimal number of a form F depend on the given field as can be easily seen by considering binary cubic forms over the real and complex fields. The minimal number and the factorization rank of a form F are invariant under non-singular linear transformations in a given field. Elsewhere³ the author defined ranks of F and the matrix B associated with F for every grouping of the indices of F into signant partitions, where there are an even number of signant partitions. These ranks were obtained by considering generalized determinant minors of matrices made up of blocks equal to B . It has been proved elsewhere⁶ for binary forms and any

field that all ranks with two partitions signant are equal. Simple considerations yield the following theorem concerning the equality of ranks valid for fields for which the matrix of F is unique.

THEOREM 5.1. *The ranks of F are equal for a field ϕ if and only if the factorization rank and minimal number of F with respect to ϕ are equal.*

A limited subclass of the class of ranks introduced by the author was defined and studied by Hitchcock.⁷ The author extended this class³ to essentially the largest possible class with the hope that this enlarged class would completely characterize classes of equivalent multilinear forms for arbitrary fields. The class of ranks of a given form contains a finite number of elements. Since the author recently proved⁸ that the number of classes of equivalent trilinear forms for non-denumerably infinite fields is non-denumerably infinite, it is impossible to characterize these classes by means of ranks alone; and it is certainly impossible to characterize classes of equivalent ordinary forms by ranks only. It is to be expected that these ranks characterize the classes of equivalent forms for certain finite fields, but this problem has not yet been studied for the general case.

6. *Concluding Remarks.*—By the well-known transition from non-homogeneous polynomials to forms it is evident that many of the theorems [e.g., theorem 1.1] may be stated for ordinary polynomials. The proofs of the remarks and theorems of this paper as well as applications to the problem of factorability of polynomials will appear elsewhere.

¹ Oldenburger, *Bull. Amer. Math. Soc.*, **42**, 871–873 (1936).

² Oldenburger, *Trans. Amer. Math. Soc.*, **39**, 422–455 (1936).

³ Oldenburger, *Ann. Math.*, **35**, 622–657 (1934).

⁴ For uses of such determinants involving variables the reader is referred to the following papers:

Oldenburger, *Bull. Amer. Math. Soc.*, **38**, 385–387 (1932).

Oldenburger, *Amer. Jour. Math.*, **59**, 427–435 (1937).

Oldenburger, *Bull. Amer. Math. Soc.*, **43**, 546–553 (1937).

⁵ Hocevar, *Compt. Rend.*, **138**, 745–747 (1904).

⁶ Oldenburger, *Ann. Math.*, **38**, 172–177 (1938).

⁷ Hitchcock, *Jour. Math. and Phys.*, **7**, 40–79 (1927).

⁸ Oldenburger, *Duke Math. Jour.*, **2**, 671–680 (1936).

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

May 15, 1938

Number 5

Copyright 1938 by The National Academy of Sciences

RELATIVE NUMBERS OF OPERATORS AND SUBGROUPS OF A FINITE GROUP

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated March 19, 1938

Operators and subgroups are the fundamental constituents of a group. The former are commonly called the elements of a group while the latter furnished the first important entering wedge into the theory of groups when J. L. Lagrange (1736–1813) proved that the order of the symmetric group of every given degree is divisible by the order of each of its subgroups. Sometimes the number of operators of a given group is larger than the number of its subgroups if by subgroup we mean a proper subgroup, that is, a subgroup which is neither the identity nor the group itself. The term subgroup will be used with this meaning throughout the present article. It is evident that in every group of finite order the number of operators exceeds the number of its cyclic subgroups since an operator of highest order of such a subgroup cannot appear in any cyclic subgroup of the same order besides the one which it generates. In particular, every cyclic group involves more operators than subgroups.

An elementary category of groups in which the number of operators of each group is always less than the number of its subgroups is composed of the abelian groups of order 2^m , $m > 2$ and of type 1^m . In fact, the number of the subgroups of index 2 in such a group is equal to the number of its operators of order 2 according to a well-known theorem. In the special case when $m = 3$ this group contains 8 operators and 14 subgroups. It is easy to verify that every other group whose order is less than 16 contains at least as many operators as subgroups. In the dihedral group of order 2^m the number of subgroups is readily found to be equal to $2^m + m - 3$. Hence the number of its subgroups is equal to the number of its operators when $m = 3$ but for all larger values of m the number of subgroups exceeds the number of operators. We proceed to consider the relative numbers of the substitutions and subgroups in the symmetric group of degree n .

It is easy to verify that the symmetric group of degree 4 contains 28 subgroups while it contains only 24 substitutions. To prove that the

symmetric group of degree $n > 4$ contains more subgroups than substitutions it is therefore only necessary to prove that the symmetric group of degree n must have this property whenever the symmetric group of degree $n - 1$ has it. Since the number of the subgroups and of the substitutions of degree $n - \alpha$ which appear in the symmetric group of degree n is n/α times the numbers of these subgroups and substitutions in the symmetric group of degree $n - 1$ and the number of the subgroups of degrees 2 or 3 in any symmetric group is then equal to the number of the substitutions of these degrees, respectively, the theorem in question will be proved if we can prove that the number of the subgroups of degree n in the symmetric group of degree $n > 4$ is at least equal to the number of substitutions of this degree increased by one.

From the fact that if the number of subgroups in the symmetric group of degree $n - \alpha$ exceeds the number of the substitutions in this symmetric group it results that the number of the subgroups of degree $n - \alpha$ in every larger symmetric group exceeds the number of its substitutions of this degree by a number which is at least equal to the degree of the symmetric group. This results directly from the well-known number theory theorems that the product of any k successive positive numbers is divisible by $k!$ and exceeds by at least k the product of any $k - 1$ successive positive numbers divided by $(k - 1)!$ whenever the smallest of these successive numbers exceeds unity and $k > 1$. In particular, from the fact that the number of subgroups of degree 4 in the symmetric group of this degree exceeds by 4 the number of its substitutions it results directly that the number of the subgroups of degree 4 in the symmetric group of degree $n > 4$ is at least $4n$ times the number of its substitutions of degree 4.

The symmetric group of a prime degree p contains more substitutions of order p than subgroups which separately involve an invariant subgroup of order p or are themselves of this order. The number of the latter is equal to the number of the different divisors of $p - 1$, including the identity and $p - 1$, multiplied by $(p - 2)!$, while the number of the substitutions of order p is $(p - 1)!$. This symmetric group contains a large number of subgroups of degree p which do not involve a substitution of degree p . In fact, every symmetric group of degree n contains subgroups of degree n which do not separately involve substitutions of this degree whenever n exceeds 4. Such groups are necessarily intransitive since every transitive group of degree n is known to contain at least $n - 1$ substitutions of this degree in view of the fact that the average number of letters in all of its substitutions is $n - 1$. The groups of degree 5 which involve no substitution of this degree are formed by establishing a 3,1 isomorphism between the symmetric group of degree 3 and the regular group of degree 2.

The number of these groups is 10 since the symmetric group of degree

5 contains 10 symmetric groups of degree 2. If a group of degree 6 does not involve a substitution of this degree it contains three transitive constituents of degree 2 and hence it is of order 4 and is invariant under the group of degree 6 and of order 48. There are therefore 15 such groups in the symmetric group of degree 6. From the two given categories of groups, it follows directly that every symmetric group of degree $n > 4$ contains groups of degree n which do not separately contain substitutions of this degree since we can readily form direct products which have this property and involve one of the given groups in each case. In particular, the symmetric group of degree $p > 3$ contains more subgroups which either involve separately a cyclic subgroup of order p or involve no substitution of degree p but have transitive constituents of degree $p - 2, 2$ as it has substitutions of degree p . That is, the symmetric group of prime degree $p > 3$ contains more than $(p - 1)$ subgroups which separately either involve invariantly a subgroup of order p or have two transitive constituents of degrees $p - 2, 2$, respectively, and involve no substitution of degree p .

To complete a proof of the theorem that the symmetric group of degree n contains more subgroups than substitutions whenever $n > 3$ it remains only to consider the group which contains a cyclic substitution of degree n when n is not a prime number since to all other cases we can apply the theorem that there are more subgroups than substitutions of degrees less than n . This results from the fact that the substitutions and the subgroups of such degrees are contained in the direct products of substitutions and of groups of lower degrees. It may also be noted that in the groups obtained by extending a cyclic group of degree $n - 2$ by substitutions on these letters which transform it into itself and by making the holomorph thus obtained isomorphic with the group of degree 2 by dimidiation, the substitution of degree n and of order less than $n - 2$ need not be considered since they appear in direct products of groups whose constituents are all of a lower degree than $n - 2$. Hence there results the theorem that *every symmetric group whose degree exceeds 3 contains more subgroups than substitutions.*

It was noted above that the number of the subgroups of the abelian group of order 2^m and of type 1^m exceeds the number of its operators whenever $m > 2$. It may be noted that in the abelian group of order p^m and of type 1^m the number of operators exceed the number of subgroups for every given value of m provided the prime number p exceeds an arbitrary given number. This results directly from the well-known formula for the number of these subgroups since the power of p in the numerator is larger than in the denominator whenever the subgroups are proper subgroups. For instance, when $p = 3$ and $m = 3$ there are 26 subgroups and 27 operators, but for larger values of m the number of subgroups exceeds the number of operators when $p = 3$.

MINIMUM DEGREE OF SUBSTITUTIONS OF HIGHEST DEGREE IN A GROUP

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated April 15, 1938

If a substitution group G of degree n has k transitive constituents then it involves at least one substitution whose degree exceeds $n - k$ since the average number of letters in all of its substitutions is $n - k$ and the identity involves no letters. The maximum value of k is $n/2$ and when k has this value each of the transitive constituents of G is of degree 2 and G is abelian. When n is odd the maximum value of k is $(n - 1)/2$ since at least one of the transitive constituents is then of odd degree and this degree is at least as large as 3. As G contains at least one substitution whose degree exceeds $n - k$ the minimum value of the degree of its largest substitutions is $n/2 + 1$. When the largest degree of a substitution of G is $n/2 + 1$ then all the transitive constituents of G are of degree 2 and $n/2$ is odd since this largest degree must be even. That is, *when a substitution group of degree n involves a substitution of degree $n/2 + 1$ but no substitution of a larger degree then $n/2$ is odd and each of the transitive constituents of the group is of degree 2.*

To construct such a system of substitution groups let n be of the form $2^m + 2^{m-1} + \dots + 2$ and hence $n/2 + 1 = 2^m$. When $m > 1$ the group generated by a substitution of order 2 and of degree 2^m is extended by a substitution of order 2 and of degree 2^m which has 2^{m-2} of its cycles of order 2 in common with the preceding substitution. There results a group of order 4 and of degree $2^m + 2^{m-1}$ which involves only substitutions of degree 2^m besides the identity. It should be noted that there are three sets of 2^{m-2} cycles which always appear together in these three substitutions whenever such a substitution involves one of them. When $m > 2$ this group of order 4 is extended by a substitution of degree 2^m which has 2^{m-3} cycles in common with each of the given three sets of 2^{m-2} cycles. Thus there results a group of order 8 which contains seven sets of 2^{m-3} cycles which always appear together and involves only substitutions of degree 2^m besides the identity.

When $m > 3$ this process can be continued until the given sets of cycles are composed separately of a single cycle. It therefore results that whenever n is of the form $2^m + 2^{m-1} + \dots + 2$ there is an abelian group of degree n whose substitution of largest degree is of degree $n/2 + 1 = 2^m$ and whose order is 2^m , which involves only substitutions of degree 2^m besides the identity. This group contains the same number of substitutions of every degree as the regular abelian group of order 2^m and of type 1^m and it coincides with this regular group when $m = 1$ and only then.

Each of its letters appears in exactly one-half of its substitutions. In general, the order of G cannot be less than $n/2 + 1$ since it must involve at least $n/2$ substitutions of degree $n/2 + 1$ in order to bring the average number of letters to $n/2$ on account of the identity, because the degree of no substitution can exceed the average degree by more than one.

When the order of G is $n/2 + 1$ then G belongs to the system of groups defined above since each of its substitutions besides the identity is then of degree $n/2 + 1$. Hence each of these $n/2$ substitutions has then exactly half of its letters in common with every other one. If we start with such a substitution the second substitution can be selected in essentially only one way and the same remark applies to every additional generator whenever the generators of a proper subgroup have been found. It should be observed that each of the successive generators are thus selected in such a manner that the group generated by it and the preceding generators is of the largest possible degree. Every G in which the substitution of highest degree is of degree $n/2 + 1$ is generated by its substitutions of highest degree since each of the substitutions which is not of highest degree involves less than the average number of letters of the substitutions of G . In particular, the substitutions of degree $n/2 + 1$ are positive.

Suppose now that the order of G exceeds $n/2 + 1$ and select an arbitrary substitution s of G which is of degree $n/2 + 1$. Consider the set of all the substitutions of G which have the property that their products into s are all of degree $n/2 + 1$. The number of these substitutions exceeds $n/2$ since G involves at least two substitutions whose degrees are less than $n/2 + 1$. Not more than $n/2 - 1$ of these substitutions can be of degree $n/2 + 1$ for if more than $n/2 - 1$ would be of this degree then at least two of them would have the same half of their letters in common with s while their other letter would be different from those in s . The product of these three substitutions would have a larger degree than $n/2 + 1$, which is impossible.

For each of the remaining substitutions of the given set there would be more than one substitution in G of degree $n/2 + 1$, since a substitution of degree $n/2 - 1$ would be negative, while the product with s would give only one such substitution. As this is impossible it has been proved that *a necessary and sufficient condition that there is a group of degree n such that its substitution of largest degree is of degree $n/2 + 1$ is that n is of the form $2^m + 2^{m-1} + \dots + 2$ and there is one and only one such group for an arbitrary given value of m .* All the substitutions of this group besides the identity are of degree $n/2 + 1$. There is therefore one and only one group whose order is a given power of 2 which has the property that it can be represented as a substitution group of degree n whose substitution of largest degree is $n/2 + 1$. The degrees of these groups are 2, 6, 14, etc.

It has been noted that when the degree n of G is odd then its substitu-

tions of largest degree cannot have a degree which is less than $n/2 + 3/2$ and it is easy to see that when $n = 3$ the symmetric and the alternating groups involve such substitutions and when $n = 5$ there is a group of order 6 whose transitive constituent groups are of degrees 2 and 3, respectively, and whose substitutions of largest degree are of degree 4. By means of what precedes it is easy to prove that these three groups are the only ones whose substitutions of largest degree are of degree $n/2 + 3/2$. Such a group involves one and only one constituent group of degree 3 and the other constituent is an isomorphism between groups of degree 2. As the order of the latter constituent exceeds 2 when $n > 5$ its substitution of largest degree is at least $\frac{n-3}{2} + 1$ and the substitution of largest degree in G is therefore greater than $n/2 + 3/2$.

MEASUREMENTS OF RESISTANCE AND CAPACITY OF MONOFILMS OF BARIUM STEARATE

BY C. E. BUCHWALD, D. M. GALLAGHER, C. P. HASKINS, E. M. THATCHER
AND P. A. ZAHL

HASKINS LABORATORY, UNION COLLEGE

Communicated March 24, 1938

In the course of a study of the modification of surfaces formed from barium stearate monolayers by superposed films composed of less than a single complete layer of molecules, undertaken at the suggestion and with the help of Dr. Irving Langmuir, it became desirable to make some rather careful measurements of the electrical resistance and capacity of barium stearate monolayers. This work has proven sufficiently interesting and suggestive as to be worthy of preliminary announcement at this time. Further work is in progress, and a more complete paper will be published shortly.

Attempts were at first made to measure electrical resistance of barium stearate monofilms by direct means, using for this purpose electrodes consisting of heavily chromium plated and polished brass slides upon which the films were mounted by standard dipping methods. Both electrodes were coated with the number of films to be measured, and were immersed in dilute copper sulfate as electrolyte, the cell thus formed being inserted in the arm of a d. c. bridge of conventional design. It was found that polarization effects were of such magnitude as to render such a method nearly valueless.

Recourse was next had to alternating current methods. A cell of the design described above was made a part of a conventional a. c. bridge network, 0.25 volts at 1000 cycles being impressed across the electrodes, and resistance and capacity measurements being recorded together. This arrangement, although workable, was undesirable in several respects. For mathematical reasons involved in the calculation of a. c. networks, it

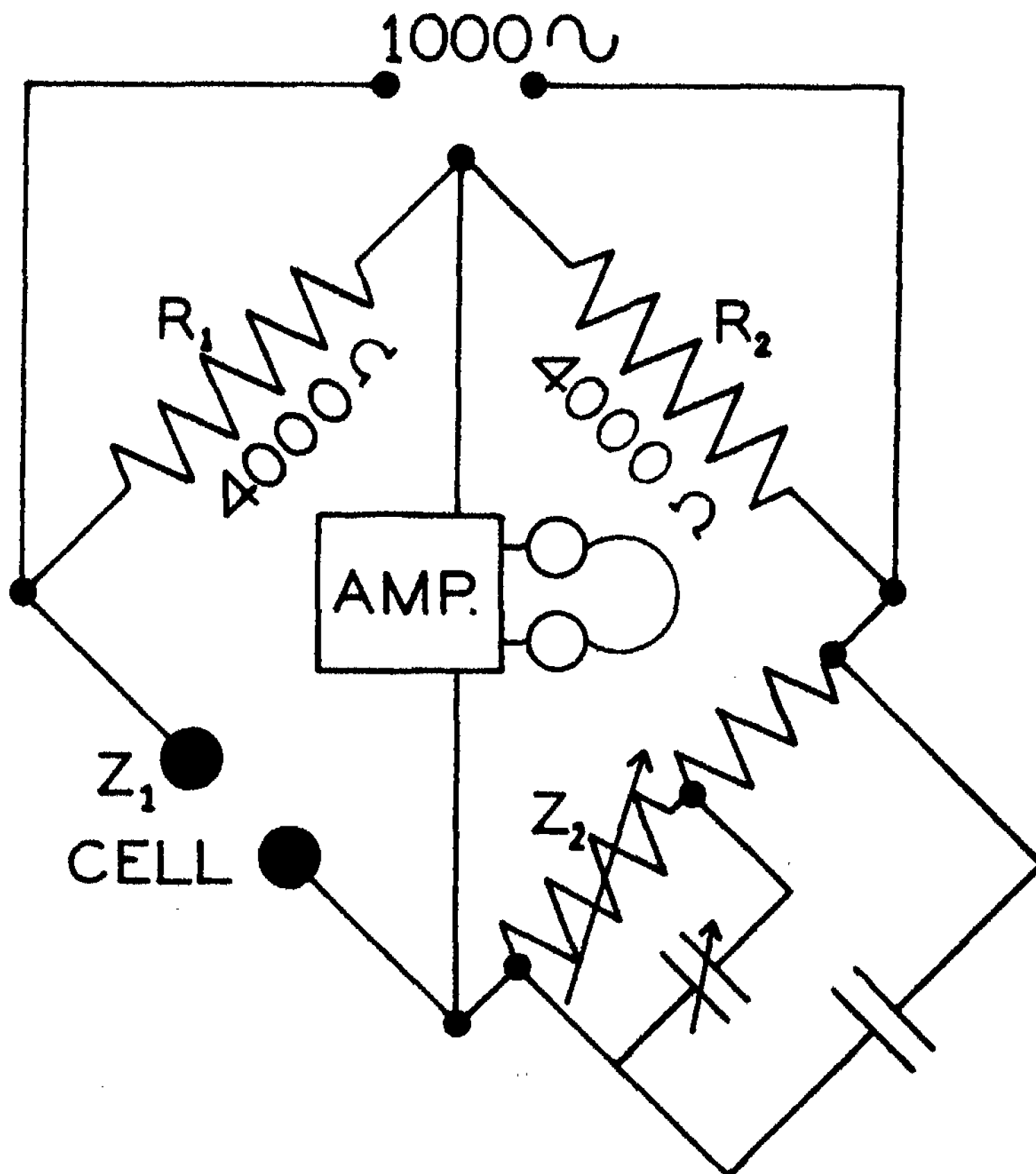


FIGURE 1

proved impracticable to obtain absolute readings for either resistance or capacity from the set, and the curves obtained showed a modification of shape which could be accounted for on grounds entirely apart from the structure of the films. Further, the form of the electrodes was not satisfactory, the chromium plating at the edges presenting sufficiently roughened surfaces so that it is highly probable that the first or subsequent layers showed irregularities or "holes" in these areas—a condition which

we have shown to be fatal to accurate measurement, since each "hole" acts as an extremely wide-mouthed potential funnel. Further, it was undesirable that the current density should be highly variable over the surfaces of the films, as it clearly must be where edges and corners are present in the electrode.

For these reasons, the arrangement finally adopted for measurement was somewhat different. The network used is indicated in figure 1. It will be seen that the circuit used constitutes a standard a. c. bridge, with the exception that an auxiliary circuit, involving a variable capacity and resistance, has been introduced into the arm Z_2 . This allows a preliminary balancing of the bridge against the *unfilmed* cell chromium CuSO_4 -chromium, and, as we shall indicate in detail in a later paper, permits direct determination of the capacity and resistance of the films laid down. As in the earlier bridge, of which this was a modification, 0.25 volts were

TABLE 1
RESISTANCES AND CAPACITIES OF BARIUM STEARATE FILMS

(These values and those shown in figure 2 are given as direct readings with both electrodes filmed. The capacities for single layers are double and the resistances one-half of the values given.)

NO. OF LAYERS	RES.	CAP. μ	RES.	CAP. μ	RES.	CAP. μ	RES.	CAP. μ	RES.	CAP. μ
1 (B)	10	6.84	10	6.42	11	6.73	23	2.71	19	2.82
3	46	3.98	51	3.99	51	3.97	42	3.33	49	3.41
5	96	2.79	92	2.98	99	3.01	71	3.02	89	2.07
7	176	2.01	193	2.07	184	1.58	101	2.71	208	1.42
9	261	1.61	271	1.53	273	0.99	132	2.15	381	0.93
11	390	1.26	403	0.97	392	0.86	239	1.40	489	0.71
13										
15	931	0.896	807		730	0.58			834	0.52
17										
19										
21	1581	0.501	1350	0.381	1353	0.39	801	0.560	1299	0.390
31	2731	0.315	2501	0.263	2638	0.234	1811	0.251	2108	0.304
41	3710	0.218	3612	0.198	3920	0.109	2847	0.151	3112	0.241

impressed across the cell at 1000 cycles. Current density was therefore low throughout the cell, a feature highly to be desired in guarding against film modification or rupture during the measurements. Standard General Radio and Leeds and Northrup variable precision condensers, backed by a battery of fixed standard condensers of 1 mfd. capacity, and standard Leeds and Northrup variable resistances were used in the balance arms. A storage battery was used as the voltage source, and readings were made with head phones, a 1000 cycle oscillator being used. Voltage wave-form and amplitude were checked continuously with a Type TMV-122-B R. C. A. cathode ray oscillograph.

The design of electrode finally adopted consisted of a cylindrical brass rod, 1 centimeter in diameter and 30 centimeters long, heavily and uni-

formly chromium plated and thoroughly polished. The end of the rod was of the form of a hemisphere, and was so joined to the cylinder that no discontinuities of surface whatever were presented. There was thus made available a surface upon which monofilms might be mounted which presented no sharp edges, corners or discontinuities, and from which water might drain evenly when dipping. The electrodes were separated by suitable bakelite collar mountings, were both filmed as before, and were mounted, as before, in an electrolyte bath of 0.35 M CuSO_4 . Connection was made with the electrodes by means of spring clips.

A standard Langmuir tray was used for preparing the films. The bath contained 100 mgm. $\text{BaCl}_2 \cdot 6 \text{H}_2\text{O}$ in 4450 cc. distilled H_2O and was

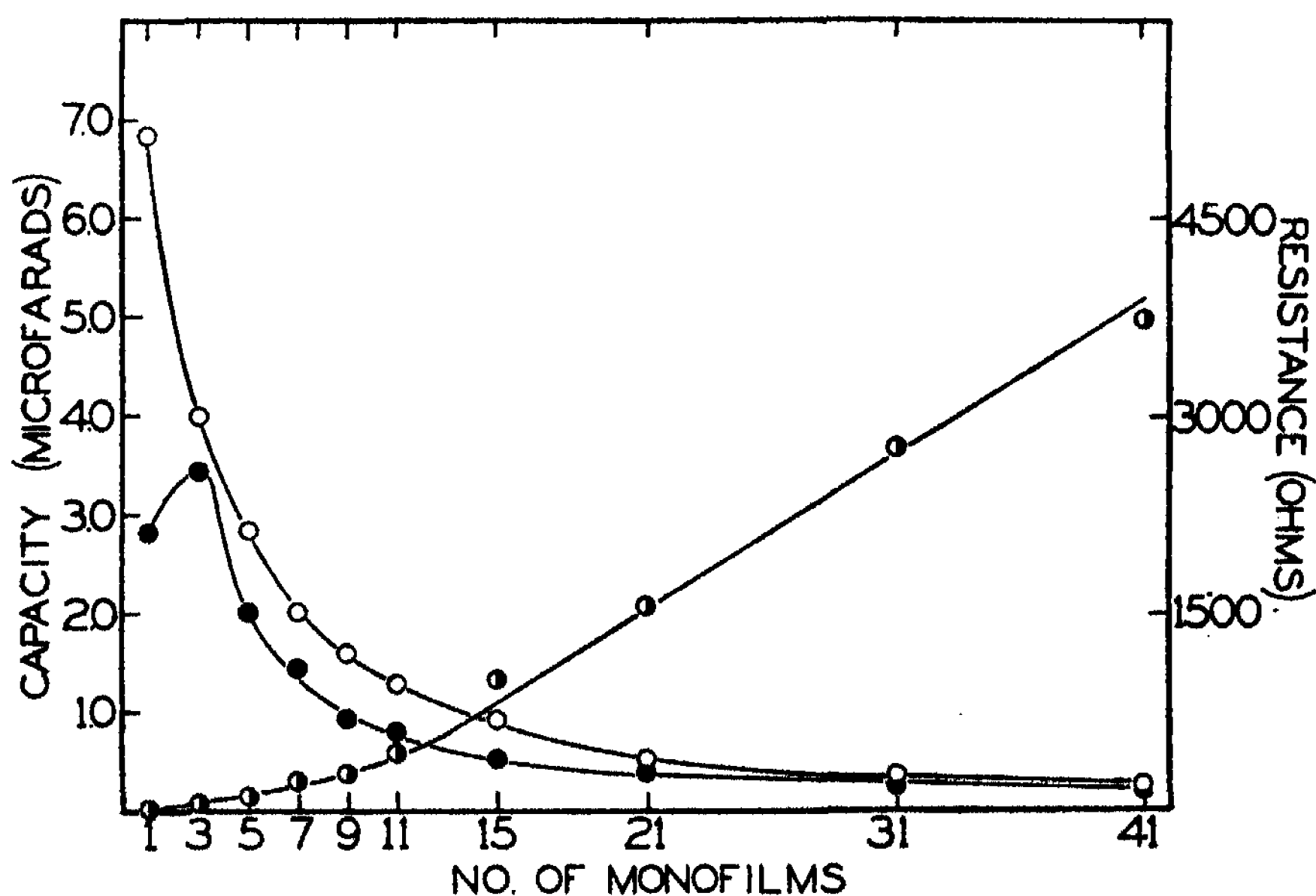


FIGURE 2

of pH 7.31. Stearic acid, from a solution of 142 mg. stearic acid in 40 cc. of redistilled benzene, was spread in standard fashion; oleic acid being used as piston oil. The electrodes were dipped by hand, and dipping was done with extreme care, as the slightest imperfection in the first B film proved serious.

The results are shown in table 1. Here are listed the resistances and capacities of varying numbers of film layers for five sets of runs. It will be noticed that in three of these runs the highest value for the capacity is shown for the initial (B) layer. For the remaining two, the layers deposited on the *second* dip (total, BAB) show a higher capacity value than does the original B. This anomaly has been studied rather carefully, and

it has been found that, when a peak of capacity thus occurs, the capacity values for numbers of layers greater than three are atypical. This is shown rather clearly in figure 2. Here are plotted the values for the resistance and the capacity as a function of number of layers laid down for a cell in which the B layer showed the highest value for capacity, and which otherwise behaved normally. There is shown on the same plot, in broken line, the capacity-layers curve for a cell in which a peak of capacity occurred at the BAB reading. It will be noticed that the curve is subsequently divergent from the typical one, which it, however, approaches as the number of layers mounts. The interpretation of this situation will be presented later.

Work is in progress on this problem, and it is hoped soon to publish more detailed results and interpretation.

TRANSLOCATION IN RELATION TO MOSAIC FORMATION IN MAIZE

BY DONALD F. JONES

CONNECTICUT AGRICULTURAL EXPERIMENT STATION

Communicated March 28, 1938

Paired changes in the developing endosperm of maize seeds show clearly that these alterations are not due to gene mutation or loss but to an unequal mitosis of such a nature that dominant genes are removed from one daughter cell and increased in the other. This inequality in somatic cell division may be brought about by crossing over in a multiple strand stage or by some form of segmental shift or interchange between homologous or non-homologous chromosomes.

The possibility of somatic crossing over in maize was looked for in the following way. Plants of the composition $C\ wx$, both on chromosome 9, were pollinated by plants having $c\ Wx$. The resulting triploid tissue in the endosperm had the genetic composition of $C\ C\ c\ Wx\ wx\ wx$. Shifts of the dominant C were expected to show as paired light and dark spots. In 903 seeds examined 113 light spots were found some of which were clearly paired with areas darker than normal. These seeds were cut with a file and stained with iodine. If there had been an exchange of segments between chromatids of homologous chromosomes the dark part of these aleurone twin spots should be underlaid with waxy tissue staining red in contrast to the blue-staining normal starch whenever the segment exchanged included both C and wx . Previous examination of similar mosaic areas has shown the removal of both of these linked genes in about half

of the cases. In all of the seeds examined no waxy tissue was found coinciding with the dark aleurone part of the twin spot. This indicates that either the *C wx* segment was lost or exchanged with some other non-homologous chromosome.

Genetic evidence previously given indicates that there is an interchange between non-homologous chromosomes in the endosperm tissue of maize (Jones, 1937). This reciprocal exchange is shown by paired spots, one part of which exhibits the loss of one dominant gene, the other and adjoining part exhibits the loss of another dominant gene, the two genes located on different chromosomes, but both removed simultaneously. This result could be brought about either by a reciprocal translocation or by a transposition of one part of a chromosome to another followed by the loss of the augmented chromosome. In a few cases there is evidence to show that the gene removed from one daughter cell is added to the other and the reverse, that is, a reciprocal interchange.

By using genes in different chromosomes that are easily followed in somatic cells the following additional results were obtained in one family having 1076 seeds grown without any treatment to produce aberrations.

GENES USED AS MARKERS		NUMBER AND CHARACTER OF SPOTS SHOWING REMOVAL OR EXCHANGE OF		
FIRST	SECOND	FIRST GENE	SECOND GENE	BOTH GENES
<i>C</i>	<i>Pr</i>	270 White	170 Red	6 White and Red
<i>C</i>	<i>Su</i>	270 White	21 Sugary	1 White and Sugary
<i>Pr</i>	<i>Su</i>	170 Red	21 Sugary	0 Red and Sugary

The mosaics showing removal or exchange of both genes are paired, adjacent areas each part of which exhibits the action of the recessive allele of the gene stated. In the one white and sugary paired spot found the sugary area underlaid a dark aleurone area of similar outline. The colorless area was underlaid by waxy endosperm. This shows that a segment of the left arm of the number 9 chromosome containing *C* and *Wx* was exchanged for a segment of the right arm of the number 4 chromosome containing *Su*. In many of the red and white paired spots the red area has a darker color than the unpaired red spots on the same seeds. This indicates that there has been an exchange of *C* and *Pr* and not a loss of both in adjacent cells.

The genes used are: *C c* determining aleurone color; *Pr pr* differentiating between purple and red; and *Su su* controlling the conversion of sugar into starch. The removal of any one of these dominant genes, if present singly, can be detected in small areas, in many cases in single cells. The *su* tissue in small areas can be seen only after cutting and staining with iodine.

Taking all the data so far obtained, including mosaics in which these genes are involved, the following results are tabulated:

GENES USED AS MARKERS		NUMBER AND CHARACTER OF SPOTS SHOWING REMOVAL OR EXCHANGE OF		
FIRST	SECOND	FIRST GENE	SECOND GENE	BOTH GENES
<i>C</i>	<i>Pr</i>	9627 White	1571 Red	54 White and Red
<i>C</i>	<i>Su</i>	270 White	21 Sugary	1 White and Sugary
<i>Pr</i>	<i>Su</i>	929 Red	100 Sugary	0 Red and Sugary

These results are combined from many families and all three genes were not heterozygous in all of them. But the ratio of single losses to double gene exchanges or losses in paired areas can be compared and this ratio derived from the above figures is as follows:

<i>C</i>	<i>Pr</i>	<i>C Pr</i>
178	29	1
<i>C</i>	<i>Su</i>	<i>C Su</i>
270	21	1
<i>Pr</i>	<i>Su</i>	<i>Pr Su</i>
186	20	0

The number of somatic changes involving a single gene as listed include both paired and unpaired changes of that gene. Both *C* and *Pr* produce dark areas when present in increased numbers but whether paired or not each mosaic results from a removal of a gene from one cell. Comparing the frequency of single gene removals in the same individuals we see that *C* is taken out about 6 times as frequently as *Pr* and about 13 times as frequently as *Su*. *Pr* is lost about 9 times more often than *Su*. But the ratio of simultaneous losses of two genes in different chromosomes to the separate losses of the individual genes involved is more nearly constant.

In this triploid tissue there are 30 somatic chromosomes. Presumably segmental interchange may take place between either arm of any two chromosomes. If this exchange takes place at random 1 in 58 mosaic areas would be expected to show the removal of any two heterozygous genes in different chromosomes. The result would be paired mosaics showing the action of recessive genes in approximately equal and adjoining areas. Any exchange that does not include both genes would be classed as a single mosaic. The relative frequency of single mosaics is determined possibly by the position of the gene on the chromosome. In the three inter-chromosomal gene pairs considered, one gene is removed more frequently than the other. Therefore it would be expected that the ratio of paired losses to single losses would be less than 1 to 58 for one gene and more than 1 to 58 for the other. This is in agreement with the observed results in two of the three cases shown.

No paired losses of *Pr* and *Su* were found in this untreated material. In seeds x-rayed shortly after fertilization 15 such mosaic areas were noted in 560 seeds examined. In the treated material the single gene losses

were so numerous and complex in outline that no comparative counts could be made. Since no *Pr Su* paired mosaic areas were observed in 4117 untreated seeds examined where at least 5 were expected they may have been overlooked.

That these gene shifts occur simultaneously is shown by the fact that the paired areas are approximately equal in area and outline and in a few cases have been seen to include only two cells. In several instances multiple changes have been found showing three or more adjoining areas of approximately equal size. Some of these triple spots are red, white and dark; sugary, white and dark; and light, white and dark. In these cases either more than two chromosomes are involved or additional changes occur at subsequent cell divisions. The exact nature of these changes cannot be determined at present but the cytological possibilities have been outlined by Dubinin and Khvostova (1935).

Paired and unpaired colorless and dark areas occur on the same seeds heterozygous for the *C* aleurone color gene. Either a piece of a chromosome is lost or the daughter cell that receives the extra *C* gene is non-viable, or grows in such a manner that the resulting tissue does not reach the surface where it can produce color while the other daughter cell lacking *C* does reach the surface. By following the linked waxy gene in the endosperm it is possible to demonstrate that this is the situation in a few cases but it does not seem likely that this can be the explanation for all unpaired mosaics, since unpaired dark areas are much less frequent than unpaired colorless. There is also no reason to expect cells that have received an additional dominant gene are less viable than cells that have received an extra recessive gene. Since deletions have been demonstrated cytologically to be of frequent occurrence in maize there is now good evidence to show that both deletion and translocation result in somatic changes.

If translocation takes place at random, as the evidence presented here indicates, then it would be expected to take place between homologous chromosomes as a chance recombination and in a few cases at or near homologous loci. If, in some diploid organisms, only these homologous exchanges result in viable cells or cells that are not handicapped in development the surviving visible changes would be the result of a process that simulates somatic crossing over but in reality is different from the exchange that normally takes place at germ cell formation.

Dubinin, N. P. and V. V. Khvostova, "The Mechanism of the Occurrence of Complex Chromosome Rearrangements," *Jour. Biologie*, 4, 935-975 (1935).

Jones, D. F., "Somatic Segregation and Its Relation to Atypical Growth," *Genet.*, 22, 484-522 (1937).

NOTE ON ESTIMATING BACTERIAL POPULATIONS
BY THE DILUTION METHOD

BY ROBERT DEAN GORDON

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA*

Communicated March 17, 1938

One method of obtaining an estimate of bacterial density in a solution, is to inoculate 1-ml. portions of the solution into each of several test tubes containing sterile nutrient medium; then dilute the solution in a convenient ratio and inoculate several more test tubes with 1-ml. portions, then dilute again, and so on, for several successive dilutions. After a period of incubation, the proportions of "positives" (tubes showing growth) obtained from each of the several dilutions, are taken as indications of the density of "viable" bacteria in the original solution.

In 1933 Halvorson and Ziegler¹ published a set of tables, in which they assumed the dilution ratio to be 10:1, and supposed a total of thirty test tubes to be inoculated with three successive dilutions, ten with one dilution, ten with a 10-fold greater dilution and ten with a 100-fold greater dilution than the first. If respectively p_1 , p_2 and p_3 tubes, out of the ten inoculated, show growth, their tables show a corresponding estimate of the mean density of viable bacteria in the middle dilution. For more details, their booklet¹ may be consulted.

Dr. C. E. ZoBell, of the Scripps Institution of Oceanography, in experimenting with these tables found them to be unsatisfactory and unreliable, and asked the writer to examine them. It was found that the estimates were obtained in accordance with R. A. Fisher's "criterion of maximum likelihood," which is shown to be of very questionable significance in this type of problem. In the Bayes-Laplace theory of inverse probability the estimates are modal values. Since the estimates are actually stated as three figure numbers (e.g., 2.53/ml. 43,500/ml., etc.), it seems evident that the most reasonable simple type of estimate is the *geometric mean*. The writer has developed formulas to obtain this value together with formulas for the standard deviation of its logarithm, to serve as an indication of its proportionate accuracy. It is expected to simplify these formulas to a greater degree than at present, but they are now in form which can be used for computation.

The notation of the writer is not the same as that of Halvorson and Ziegler. It is as follows:

n_{10} = number of test tubes showing growth, out of the ten inoculated with the highest concentration of the solution.

n_1 = number of test tubes showing growth, out of the ten inoculated with the middle dilution.

$n_{0.1}$ = number of test tubes showing growth, out of the ten inoculated with the highest (100-fold) dilution.

\bar{p} = geometric mean estimate of the density of viable bacteria in the solution, corresponding to the middle dilution (i.e., to n_1).

x = an integer $\geq x_0$ where $x_0 = 1110 - 100n_{10} - 10n_1 - n_{0.1}$.

$N = n_{10} + n_1 + n_{0.1}$.

γ = "digamma 1" = $-0.577216\dots$

γ' = "trigamma 1" = $+1.644934\dots$

$\log_e 10 = 2.302585\dots$

Δ = Boolean difference operator; operates on x .

H_0 = operator on x_0 .

$= (1 + E + \dots + E^{x_0})^{n_{10}} (1 + E + \dots + E^9)^{n_1}$

where E = Boolean operator = $(1 + \Delta)$;

$= 1 + P_1 E + P_2 E^2 + \dots + P_r E^r$.

With this notation, the following formulae hold:²

$$\log_e \bar{p} = \gamma + \log_e 10 - \frac{H_0 \Delta^N \left\{ \frac{\log_e x_0}{x_0} \right\}}{H_0 \Delta^N \left\{ \frac{1}{x_0} \right\}}. \quad (13)$$

$$\sigma^2_{\log_e \bar{p}} = -(\log_e \bar{p})^2 + [\gamma + \log_e 10]^2 + \gamma'$$

$$\begin{aligned} & -2[\gamma + \log_e 10] \cdot \frac{H_0 \Delta^N \left\{ \frac{\log_e x_0}{x_0} \right\}}{H_0 \Delta^N \left\{ \frac{1}{x_0} \right\}} + \\ & + \frac{H_0 \Delta^N \left\{ \frac{(\log_e x_0)^2}{x_0} \right\}}{H_0 \Delta^N \left\{ \frac{1}{x_0} \right\}}. \end{aligned} \quad (16)$$

For use in these formulas, the following auxiliary formulas have been obtained:

$$(-1)^N \Delta^N \left\{ \frac{1}{x} \right\} = \frac{N!(x-1)!}{(x+N)!}. \quad (19)$$

$$(-1)^N \Delta^N \left\{ \frac{\log_e x}{x} \right\} = \frac{N!(x-1)!}{(x+N)!} \log_e x + \quad (21)$$

$$+ \sum_{p=N}^{\infty} \frac{(-1)^{p-N+1}}{x^p + 1} \cdot \frac{p!}{p-N!} \cdot \left(1 + \frac{1}{2} + \frac{1}{3} + \dots + \frac{1}{p} \right) B_{p-N}^{(-N)}.$$

$$(-1)^N \Delta^N \left\{ \frac{\log_e x}{x} \right\} = \frac{N!(x-1)!}{(x+N)!} \log_e(x+N) \quad (21a)$$

$$- \sum_{p=N}^{\infty} \frac{1}{(x+N)^{p+1}} \cdot \frac{p!}{(p-N)!} \left(1 + \frac{1}{2} + \frac{1}{3} + \dots + \frac{1}{p} \right) B_{p-N}^{(-N)}$$

where $B_{p-N}^{(-N)}$ are Bernoulli numbers of degree $p-N$ and order $(-N)$; cf., L. M. Milne-Thompson, "Calculus of Finite Differences" (1933), page 129. We have tabulated most of these numbers which we need.

For the coefficients P_ν of the operator

$$H_0 = 1 + P_1 E + P_2 E^2 + \dots + P_\nu E^\nu + \dots$$

we have the direct formula

$$P_\nu = \sum_{r=0}^{.01\nu} \sum_{s=0}^{.10-.10r} (-1)^{r+s} C_r^{n_{10}} C_s^{n_1} C_{\nu-100r-10s}^{\nu-100r-10s+n_{10}+n_1-1} \quad (30)$$

where C_a^b is the binomial coefficient. Also

$$P_{99n_{10}+9n_1-\nu} = P_\nu.$$

For intermediate values of the subscript ν , P_ν is given by an Hermite expansion

$$P_\nu = \left[\left(\sum_{\mu=0}^{\infty} A_{2\mu} \cdot \frac{d^{2\mu}}{d\xi^{2\mu}} \right) \frac{c}{\sqrt{\pi}} e^{-c^2(\xi-b)^2} \right]_{\xi=\nu} \quad (33)$$

$$\text{where } b = \frac{1}{2}(99n_{10} + 9n_1) \quad \text{and} \quad c^2 = \frac{6}{9999n_{10} + 99n_1 + 2}$$

and the first few coefficients $A_{2\mu}$ have the following values:

$$\begin{aligned} A_0 &= 10^{2n_{10}+n_1} \\ A_2 &= 0 \\ A_4 &= - \frac{10^{2n_{10}+n_1} [(10^8-1)n_{10} + (10^4-1)n_1 + 2]}{2880} \end{aligned} \quad (38)$$

and

$$\begin{aligned} A_6 &= 10^{2n_{10}+n_1} K_6 \\ A_8 &= 10^{2n_{10}+n_1} \left[K_8 + \frac{1}{2} K_4^2 \right] \end{aligned}$$

.....

where

$$K_{2\mu} = \frac{B_{2\mu}}{(2\mu)(2\mu)!} [(100^{2\mu} - 1)n_{10} + (10^{2\mu} - 1)n_1 + 2]$$

and $B_{2\mu}$ is the Bernoulli number of first order, of degree 2μ (cf. Milne-Thompson, l. c.). A trigonometric (Fourier) expansion is also possible for P_r , and may prove more useful.

Mrs. Naomi Lancaster has used these formulas to make several computations of \bar{p} , shown in the following table and compared with the corresponding estimates of Halvorson and Ziegler:

ARGUMENTS			\bar{p}	\bar{p}	% DEVIATION
n_{10}	n_1	$n_{0.1}$	COMPUTED BY US	FROM HALVORSON AND ZIEGLER	FROM HALVORSON AND ZIEGLER
10	7	3	1.43	1.53	-7.0
8	5	1	0.291	0.267	+9.0
4	2	1	0.086	0.080	+7.5

The per cent deviations shown would appear to be significant in bacteriological work. For instance, if a bacteriologist desires to attribute a particular degree of certainty to the proposition that his estimate is within 20% of the true value, the knowledge that his method of arriving at the estimate could account for a bias of from 7 to 9% must certainly be of significance to him.

As a matter of fact, comparisons of direct plate counts with estimates from Halvorson and Ziegler's tables, on the same materials, corroborate the above theoretical comparisons and even indicate that in other parts of the tables, the bias may be considerably more serious.

In order to put these results into practical form for bacteriologists and others, as well as to test the theory experimentally, it would be necessary to compute tables to replace those of Halvorson and Ziegler,¹ which would require a considerable financial outlay.

* Contributions from the Scripps Institution of Oceanography. New Series No. 18.
¹ Halvorson and Ziegler, *Quantitative Bacteriology*, Burgess Publishing Co., Minneapolis, Minnesota (1933).
² A complete discussion will be published elsewhere.

*TEMPERATURE AND THE CRITICAL INTENSITY FOR
RESPONSE TO VISUAL FLICKER*

BY W. J. CROZIER, ERNST WOLF, AND GERTRUD ZERRAHN-WOLF

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated March 24, 1938

1. The investigation of the properties of the flicker-response contour for visual excitation¹ has involved the measurement of the inter-dependence of flash-frequency F and intensity I in a flash at the critical point for reaction signifying "recognition" of flicker. It has been shown that with very different animals there obtain relations of invariant fundamental form² between F and I , the area of receptor surface involved,³ the proportion of light-time to dark-time in the flash cycle,⁴ and the temperature⁵ of the organism. The dynamical properties of these relationships are therefore non-specific,⁶ as indeed they are for other modes and types of intensive discrimination as well.¹⁵ They are not determined by morphological characteristics of the animal as a whole,¹ nor of its peripheral receptor organs (save in minor respects not pertinent to this discussion⁷).

The one feature common to the responses concerned is their dependence upon the activity of a population of neural effects. It accords with this that a probability integral⁸ accurately describes the connection between F and $\log I$. The numerical values of the parameters of the integral are determined by the (genetic) constitution of the organism⁹, but its nature as a summation of a particular kind of probability distribution is nonspecific.

2. It is impossible to reconcile these facts, and the related phenomena of intensity discrimination in general, with the hypothesis¹⁰ that the properties of the flicker-response curve are determined by the photochemical excitability of the retina.¹¹ The parameters of the probability summation, or the parameters of any arbitrary descriptive curve which might be used, do not have the behavior required by that conception.

It is important, however, to determine what quantitative properties these parameters do exhibit. The theoretical background of the application of a probability integral to a case of this kind involves the assumption¹² that the summated neural effects appealed to are the products of elements which in time *fluctuate* in their capacity to contribute to the production of the result used as an end-point (i.e., response). On this basis the measured relationships between F_m and σ_F ¹³, I_m and σ_I ¹⁴, ΔI_m and $\sigma_{\Delta I}$ ¹⁵, etc., as functions of temperature, area, and t_L/t_D , have been extensively explored, with demonstration of their rational coherence. This is also the case with the curiously different effects of altering temperature, area and t_L/t_D , so far as the qualitative properties of the parameters of the probability integral are concerned and as regards their quantitative features¹⁶

when t_L/t_D is used as an independent variable. It was at first supposed¹⁷ that response to flicker should be so complexly determined that the data on the $F - \log I$ curve at various temperatures could not reasonably be expected to submit to simple formulation. In particular, it was suspected that the application of the activation-energy equation (Arrhenius) would not be found to yield a constant value of the "temperature characteristic."¹⁸ (This would not of itself preclude rational analysis, as I have recently shown¹⁹ for another case.)

In discussing the relation between flash frequency (F) and critical intensity (I) for reaction to visual flicker,¹⁷ under the conditions imposed by work with lower animals, we stressed the fact that for an insect (larva of *Anax*) and a vertebrate (the sunfish *Enneacanthus*) there did not appear to obtain a simple quantitative relationship between temperature and critical intensity at fixed flash-frequency. The observations were made at only three temperatures, however, and later work has led to an amplification and correction of the earlier conclusion. The effect of changing the temperature is simple enough, since it leads merely to a shift of the position of the $F - \log I$ curve on the $\log I$ axis, without changing its form or altering the maximum to which the curve rises.¹⁷ So many instances occur in which the magnitudes of events in biological systems are differently related to temperature on either side of a critical temperature²⁰ that we should have been on our guard against its happening with flicker excitations also. Where it does occur, observations at a number of temperatures are of course required to detect it.

The $F - \log I_m$ contour for the turtle *Pseudemys* is a single sigmoid curve,²¹ and not, as with other vertebrates thus far tested,¹ a complex of two (or three)²² parts. This is in keeping with the fact that its retina apparently contains no rods, but only sensory cells of a single type, cones. It should thus provide a somewhat simpler condition for analysis of the properties of the flicker-response curve as a function of temperature.

3. As with *Anax* and *Enneacanthus*, we find that the *shape* of the $F - \log I$ curve for *Pseudemys* is not affected by change of temperature. With increase of temperature the whole curve is merely moved to lower intensities. The bearing of this shift upon certain theories of the meaning of the flicker curve we have commented upon previously.¹⁷ The curve is well described by a probability integral in $\log I$.²¹ Its maximum is independent of temperature, and so is its $\sigma_{\log I}$, but the abscissa of its inflection point (τ') decreases with rise of temperature (Fig. 1).

4. From data on the measurement of I_m at any fixed flash-frequency we can then estimate the dependence of a single parameter, τ' , upon the temperature. Experiments were made at $F = 20$, and $F = 30$, the inflection point falling between them. We take $1/I_m$ as a measure of excitability, with F constant. Reasons outside the immediate considerations of

flicker phenomena also lead to this procedure.²² The excitability, in terms of marginal response to flicker, is increased with rise of temperature. Figure 2 shows that the rate of increase obeys the Arrhenius equation, with a critical temperature at 30° or a little below. The values of the temperature characteristic μ are $27,000 \pm$ (up to) 30° and $12,000 +$ (beyond 30°).

5. There are reasons for assuming that some rather direct function of the critical intensity for marginal response to flicker is a measure of the

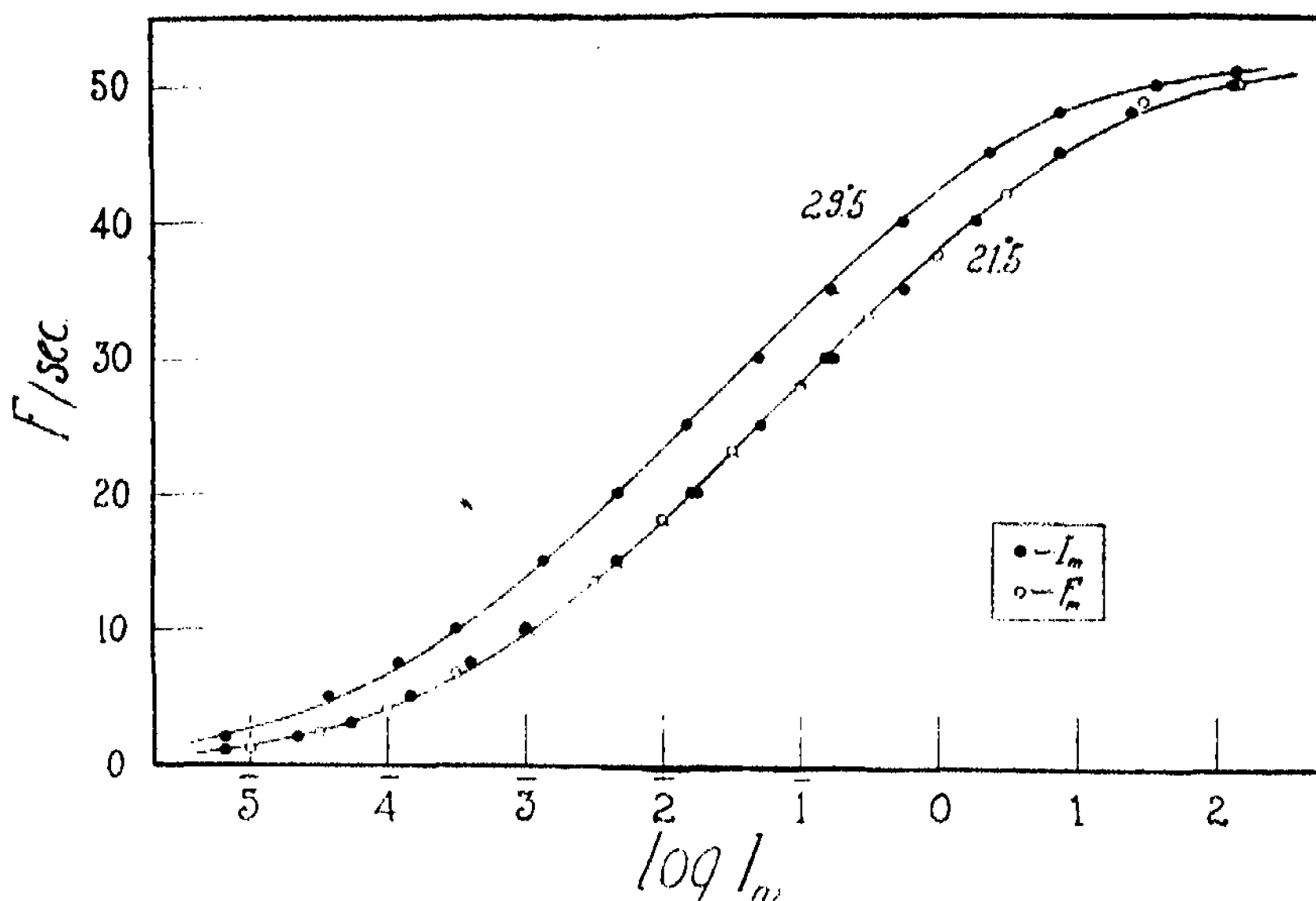


FIGURE 1

Mean critical flash illumination $t_L/t_D = 1$ for response of the turtle *Pseudemys* to visual flicker, as a function of flash-frequency F , at two temperatures. Each point is the mean¹ of three observations on each of the same ten individuals at every point. (Double determinations bear tags.) The curve is the same probability integral drawn through each series, with shift of the inflection point to the left with rise of temperature.

“driving force” responsible for the reaction. We have pointed out¹ that the relation of I_m to its variations (P.E., or σ_I) is precisely that encountered in studies of the behavior of σ_R , where R is the rate or frequency of a biological phenomenon as a function of temperature.²³ An essential fact is that the relative variation of I is constant (independent of temperature), so that σ_I/I (or $\sigma(1/I)/1/I$) is constant. The rate or frequency (as, of the heart beat) has been taken to be proportional to the velocity of a controlling chemical change. The reasons for this are (1) that it obeys the work function and (2) with a value of the “activation energy” (μ) which falls

within the range characteristic for chemical processes proceeding at ordinary temperatures. On this basis $1/I_m$ for reaction of *Pseudemys* to flicker (at fixed F) is determined by the velocity of a system of chemical transformations of which the slow member is one having $\mu = 27,000 \pm$ below 30° and $\mu = 12,000 +$ above 30° .

The obvious way in which to account for such facts is through the supposition that excitability with respect to the recognition of flicker (measured by $1/I$) is governed by the velocity of a terminal reaction affecting all the nervous elements implicated in the determination of the index

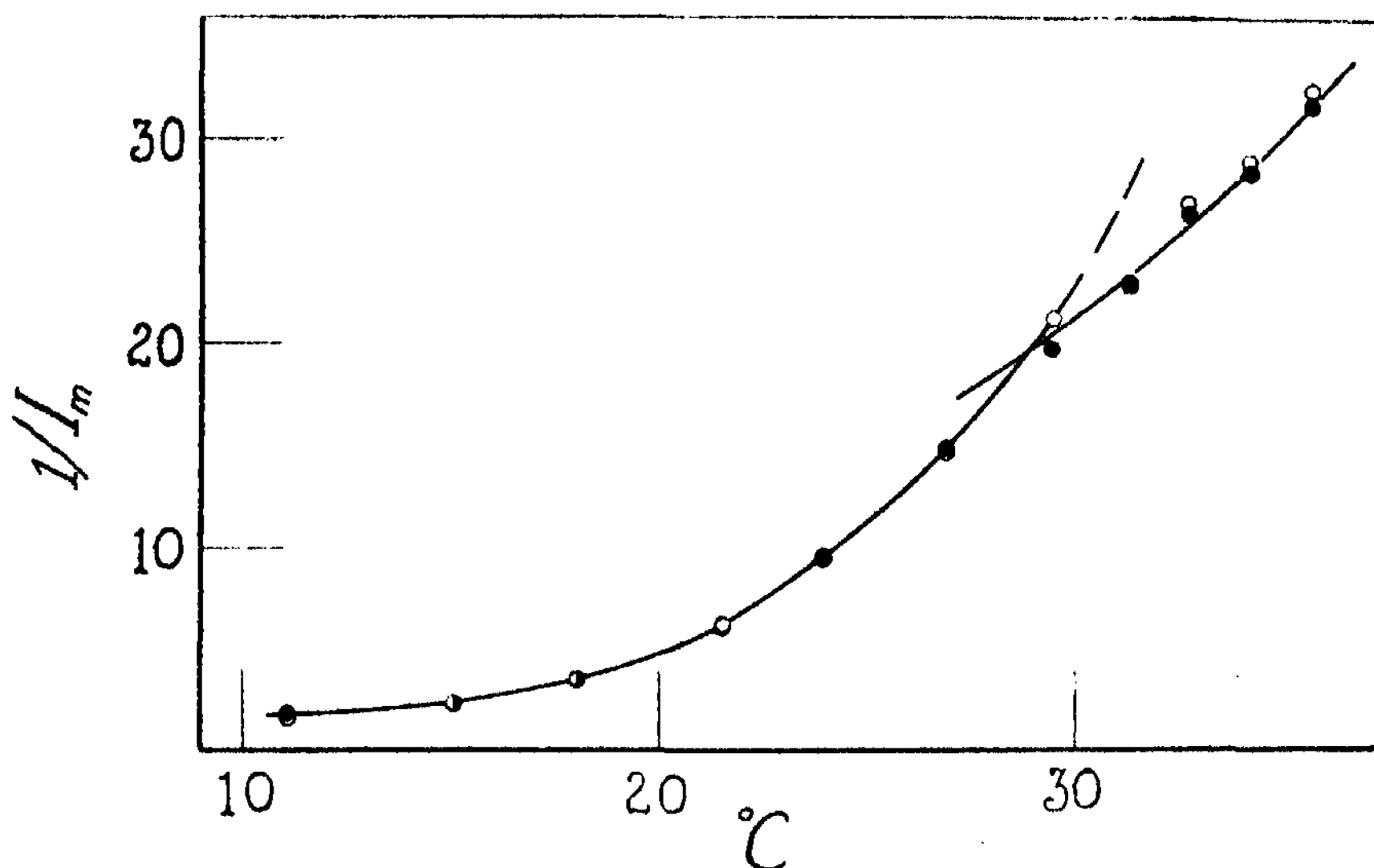


FIGURE 2

The excitability for response to flicker, measured by the reciprocal of the critical flash intensity, at fixed flash frequency, increases with temperature according to the Arrhenius equation, with a critical temperature at 30° —. Observations at $F = 20$ and at $F = 30$ are brought together for comparison by multiplying those at $F = 30$ by a constant (10.21).

response, and that the speed of this terminal reaction is controlled by a chain of reactions including at least two steps. One of these steps is characterized by $\mu = 27,000$, the other by $\mu = 12,400$.

The existence of such steps has been referred to²⁴ in terms of a catenary series. The unwarranted and irrelevant assumption has been made by some²⁵ that the links in such a chain should be, or were supposed to be, related in a mass-action sequence. The evidence has on the contrary²⁶ long required that the interconnection of the steps be in terms of catalyzed reactions, one of which "releases" the next with a frequency depending on

the speed of the first reaction. In such a chain (*a*) the frequency of occurrence of the end result depends on the slowest individual step in the chain and (*b*) the occurrence of specific, sharp critical temperatures may be rationally accounted for by the nature of the system in which these processes occur.²⁶ The association of sets of values of μ in connection with a particular phenomenon²⁴ is then reasonable if one assumes a specific organization of different catenary sets of processes in diverse situations. A mechanism for the integration of such sets of reactions in terms of the properties of catalytic surfaces remains to be more fully developed; this mechanism must of course account for the fact that critical temperatures in general are distributed in such a way as to occur predominantly at definite places on the temperature scale.²⁴ The 30° point encountered in the present experiments is one of these. The dynamics of the situation here conceived can be explored in several ways. Experiments to be described subsequently permit a decision as to whether the shift of μ at a critical temperature is due (*a*) to a modification of relations within a system of contact-catalysis processes, or (*b*) to the "injury" of a certain number of the "elements" defined by the foregoing analysis.²⁷

¹ *Jour. Gen. Physiol.*, 19, 20, 21, (1935-38).

² Crozier, W. J., *Proc. Nat. Acad. Sci.*, 23, 71 (1937).

Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, 21, 223, 17 (1937-38); *Proc. Nat. Acad. Sci.*, 23, 516 (1937).

³ Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, 21, 223 (1937-38).

⁴ *Ibid.*, 21, 313 (1937-38).

⁵ *Ibid.*, 20, 393, 411 (1936-37); and material in course of publication.

⁶ *Proc. Nat. Acad. Sci.*, 23, 71; *J. Gen. Physiol.*, 20, 393; 411; 21, 17, 203.

⁷ *Ibid.*, 20, 363 (1936-37); 21, 223 (1937-38); (in press).

⁸ *Proc. Nat. Acad. Sci.*, 23, 71 (1937); *Jour. Gen. Physiol.*, 21, 17, 313 (1937-38).

⁹ *Proc. Nat. Acad. Sci.*, 23, 516.

¹⁰ Hecht, S., *Physiol. Rev.*, 17, 239 (1937).

¹¹ *Jour. Gen. Physiol.*, 20, 393, 411 (1936-37); 21, 313; (in press) (1937-38).

¹² *Proc. Nat. Acad. Sci.*, 23, 71 (1937).

¹³ *Jour. Gen. Physiol.*, 20, 211, 363; 21, 17, etc.

¹⁴ *Proc. Nat. Acad. Sci.*, 22, 412 (1936); and footnote 1.

¹⁵ *Ibid.*, 22, 412 (1936); Crozier, W. J., and Holway, A. H., *Ibid.*, 23, 23 (1937); Holway, A. H., and Crozier, W. J., *Ibid.*, 509 (1937).

¹⁶ *Jour. Gen. Physiol.*, 21, 313 (1937-38); (in press).

¹⁷ *Ibid.*, 20, 393, 411 (1936-37).

¹⁸ *Ibid.*, 7, 189 (1924-25).

¹⁹ Cf. Crozier, W. J. (in press) and *Jour. Gen. Physiol.*, 7, 189 (1924-25).

²⁰ *Ibid.*, 7, 189 (1924-25); 9, 525 (1925-26).

²¹ *Proc. Nat. Acad. Sci.* (in press).

²² (*Triturus*) (in press).

²³ Crozier, W. J., *Déterminisme et variabilité*, Paris: Hermann, pp. 56 (1935).

²⁴ *Jour. Gen. Physiol.*, 7, 189, 429 (1924-25), etc.

¹⁶ Burton, A. C., *Jour. Cell. Comp. Physiol.*, 9, 1 (1936); see Hoagland, H., *Ibid.*, 10, 29 (1937).

¹⁷ *Jour. Gen. Physiol.* 9, 525, 531 (1925-26); 18, 801 (1934-35), etc.

¹⁸ The thermostat employed in these experiments (cf. Stier, T. J. B., and Crozier, W. J., 1932-33, *Jour. Gen. Physiol.*, 16, 757) was constructed with aid from a grant by the Elizabeth Thompson Science Fund to one of us, which is gratefully acknowledged.

SPECIFIC CONSTANTS FOR VISUAL EXCITATION. II

BY W. J. CROZIER, ERNST WOLF AND GERTRUD ZERRAHN-WOLF

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated March 24, 1938

I. In previous communications we have dealt with considerations arising from measurements of the flicker-response contours for fishes of different types, which can in certain cases¹ be subjected to genetic tests. The curves describing the relationship between flash-frequency F and flash-intensity I for various teleosts² are of specific form: their rod-excitation and cone-excitation parts are described with excellent fidelity by probability integrals (F vs. $\log I$), and the parameters of this formulation exhibit significant differences according to the kind of teleost concerned. These parameters (F_{max} ; $\log I$ at inflection; and $\sigma_{\log I}$) show properties in relation to various experimental variables which justify the use of the conception that a probability formulation is applicable to the data of response to visual flicker.³

II. Evidence has been provided² showing that the quantitative values of these parameters are dependent upon the genetic constitution of the organism.³ A test of this position, and at the same time a check upon the possible rôle of certain physiological factors which should be irrelevant if our view is correct, is provided by the determination of the $F - \log I$ curve for individuals in which an albino mutation has occurred. The test is of additional interest with respect to the significance of retinal pigmentation and of the significance of the iris of the eye.

With reference to the latter point, it could be supposed that certain quantitative properties of the $F - \log I$ curve (e.g., τ' , the abscissa of inflection) could be a function of the iris aperture, and of the translucency of the iris and ocular media. We have found no reason whatever for supposing that this is a real consideration, since the shape of the $F - \log I$ curve in neither fishes,² amphibian,⁴ turtle⁵ nor man⁶ gives any indication that it need be taken into account. It could also be supposed that in the absence of retinal melanin the relative flicker-response performance of rods and of cones could be modified in other ways. The fact that visual per-

formance curves for albino animals have not in any instance (human or otherwise)¹³ been reported upon in detail lends added importance to such observations.

The significance of an examination of this kind is of several sorts: (1) If the $F - \log I$ curve is unaltered by the albinistic state, it is shown (a) that a mutation not primarily concerned with the nature of the elements of excitability⁷ defined by the properties of the $F - \log I$ curve does not affect

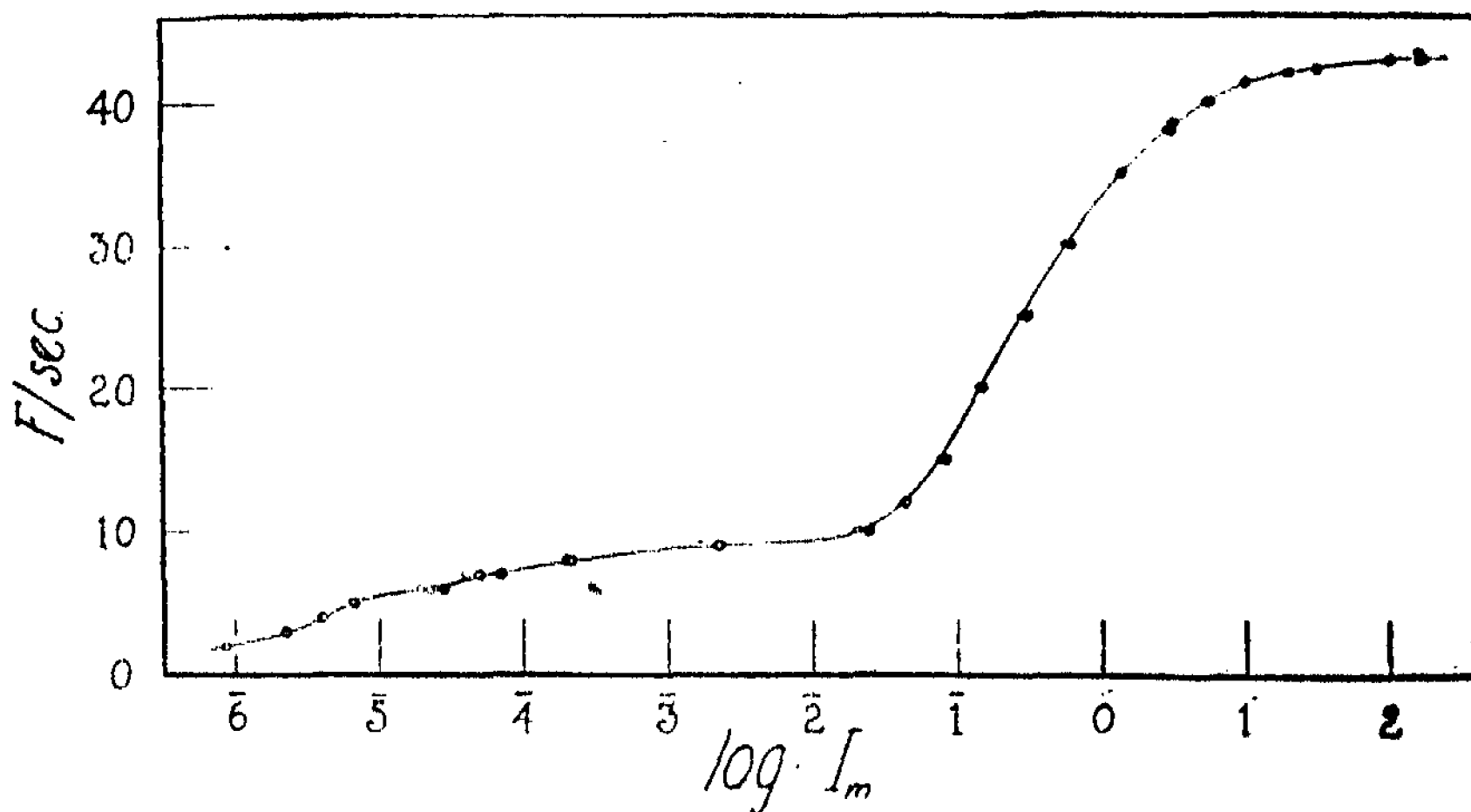


FIGURE 1

Log mean critical illumination (I_m) as a function of flash-frequency F for the teleost *Xiphophorus helleri*: open circlelets, based on 30 observations (10 individuals) of normal "wild type" as described in an earlier report;¹¹ solid circlelets and half shaded, data on three albino mutants of this stock (three observations on each at every point); 21.5°C. The "cone" curve (cf.²) for the albinos is possibly a very little beyond that for the wild type, but this is not significant. The curve drawn is the theoretically analyzed curve for the wild type.² The new data show in the zone of overlap of "rod" and "cone" contributions the kind of scatter discussed⁶ for the measurements with normal *Xiphophorus*. The half-shaded circlelets are determinations of F_m at fixed levels of I (cf.²), made to determine if for the albino individuals the curve at high intensities turns downward, as could be expected (from analogy with human data) if "glare" is an especially significant factor.

the objective measures of these properties, and (b) that melanin in iris and retina has no influence upon the $F - \log I$ curve. (2) If the curves for albino individuals should differ significantly from those of the parent stock, an interesting opportunity would be presented for further analysis.

A test of this kind has been possible with individuals of *Xiphophorus helleri*, the well-known viviparous aquarium fish. We are under obligation to Dr. C. P. Haskins of this Laboratory for the opportunity to utilize albino individuals derived from his cultures.

III. A curve of performance with respect to critical flash-illumination as a function of flash frequency has already been published⁸ for *Xiphophorus helleri*. Corresponding determinations were made with individuals arising in the same stock which were to careful inspection completely lacking in melanin, in retina and elsewhere. Three individuals (1 ♂, 2 ♀) were available for a complete series of tests.

In figure 1 it is seen that over the entire range of explorable intensities the correspondence of the measurements in the two series, normal and albino, is essentially complete. There were certain grounds for expecting that at high intensities the curve might show negative slope⁹ for the albinos, but this has not been found. The data supply a striking instance of the reproduceability of the $F - \log I$ curve. The variation of $\log I_c$ (Fig. 2.) follows the rule already found for flicker response in general,¹⁰ and shows quantitative agreement with the values gotten for non-albino individuals of this stock of *Xiphophorus*.¹¹ We have already shown that certain other genetic color differences have no influence upon the $F - \log I$ curve, but (in other genera) may influence the variability function.

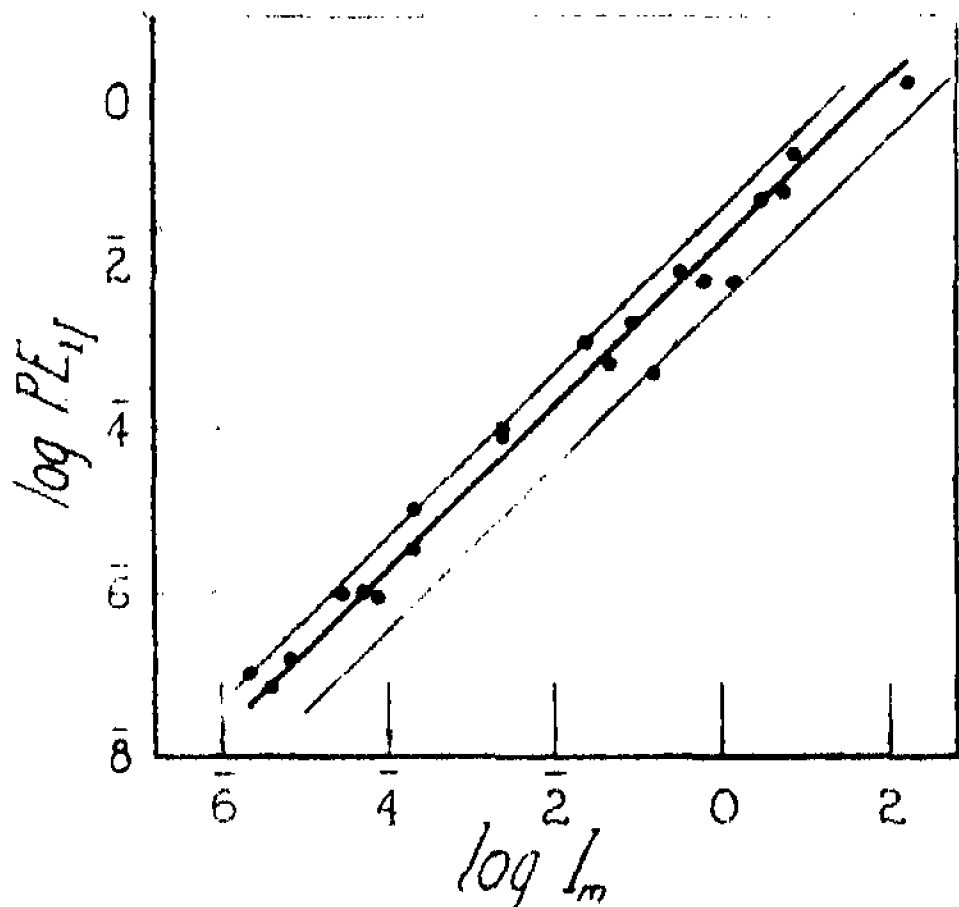


FIGURE 2

The scatter of the individual measurements of critical flash-intensity, as related to I_m , for reaction to flicker at various flash frequencies for albino *Xiphophorus helleri*. The central line drawn for mean $P.E.11$ divides the observations equally (cf.¹²). Its slope = 1, and its position is identical with that previously obtained⁸ for normal *Xiphophorus*. The breadth of the band ($= k^{\sigma\sigma}$) agrees quantitatively with that previously observed when correction is made for the fact that three individuals are involved in the present instance (albinos) and ten in the earlier series² (normals).

IV. *Summary.*—Albino individuals, mutationally arising in a stock of the teleost *Xiphophorus helleri*, show precisely the same values of critical flash illumination for response to flicker as do normal individuals of the same stock. This shows (1) that retinal pigmentation (and iris pigment) has no influence upon the quantitative performance of the implicated elements of visual excitability; and (2) that, in the absence of such superimposed influences, the $F - \log I$ curves are highly reproduceable characteristics of the composition of the organism.

¹Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Proc. Nat. Acad. Sci.*, **23**, 516 (1937); *Jour. Gen. Physiol.*, **21**, 17 (1937-38).

²Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **19**, 459 (1935-36); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **20**, 211, 411 (1935-36); **21**, 17; *Jour. Exptl. Zool.* (in press).

³Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **21**, 223; 313 (1937-38); (in press); **20**, 393, 411; *Proc. Nat. Acad. Sci.* (in press).

⁴Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Exptl. Zool.* (in press).

⁵*Jour. Gen. Physiol.* (in press).

⁶Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23**, 71 (1937); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **21**, 203 (1937-38).

⁷Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23**, 71 (1937).

Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **23**, 516 (1937); *Jour. Gen. Physiol.*, **21**, 17 (1937-38).

⁸*Ibid.*, **21**, 17 (1937-38); *Proc. Nat. Acad. Sci.*, **23**, 516 (1937).

⁹*Jour. Gen. Physiol.*, **20**, 211, 411 (1935-36).

¹⁰Crozier, W. J., *Ibid.*, **19**, 503 (1935-36); and footnotes ² and ⁴.

¹¹*Jour. Gen. Physiol.*, **21**, 17 (1937-38).

¹²Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23**, 23 (1937).

¹³Since this was written we have learned of a recent publication by Bunge, E., and Heyn, W., *Klin. Monatsbe. Augenheilk.*, **100**, 178, 1938, in which the dark adaptation curve for a human albino is shown not to differ from the normal.

THE RELATIONS BETWEEN STEFAN'S RADIATION LAW, NERNST'S HEAT THEOREM AND MAXWELL'S FORMULA FOR THE RADIATION PRESSURE

BY EUGENE GUTH AND ARTHUR E. HAAS

UNIVERSITY OF NOTRE DAME

Communicated April 15, 1938

Boltzmann, as is well known, derived Stefan's law of radiation by combining the first and second laws of heat with Maxwell's formula for the radiation pressure. On the other hand, the statement is often found that on the basis of thermodynamical relations, the empirical confirmation of Stefan's law represents at the same time an empirical confirmation of Maxwell's expression for the radiation pressure. As will be shown in the following, such a reasoning fails to be conclusive, for only by taking into account Nernst's heat theorem in Planck's formulation are we led to Maxwell's relation for the radiation pressure. Conversely, however, Stefan's law and Maxwell's formula, by their combination with the first and second laws of thermodynamics, yield Nernst's heat theorem for the special case of the black body radiation. It will be the task of the following axiomatic investigation to prove this and thus, for the special case of the

black body radiation, to determine the arbitrary functions and constants which occur in "classical" thermodynamics, based on the first and second laws.¹

We start with the energy equation of classical thermodynamics. It holds for all cases in which the state of a system is completely determined by temperature and volume, and a normal uniform pressure is the only force acting upon the system. For such cases the combination of the first and second laws yields the energy equation in the following form²

$$(\partial U / \partial V)_T = T (\partial p / \partial T)_V - p \quad (1)$$

where U , V , T and p denote the internal energy, the volume, the absolute temperature and the pressure, respectively.

If U be known as a function of T and V , we obtain p as a function of T and V , and thus the equation of state by integrating (1), a partial differential equation of the first order with respect to T . We find

$$p = T \int_0^T \left(\frac{\partial U}{\partial V} \right)_T \frac{dT}{T^2} + T f(V). \quad (2)$$

Here $f(V)$ is an arbitrary function of the volume, for the determination of which the first and second laws alone do not suffice. From the relation which represents the internal energy as a function of V and T , the equation of state of the system can thus only be derived to within an arbitrary function of the volume which is to be multiplied by the temperature.

If, on the other hand, the equation of state be given, i.e., $p = f(V, T)$, the internal energy may be derived from (1) by integrating with respect to the volume, or

$$U = \int_0^V [T(\partial p / \partial T)_V - p] dV + g(T) \quad (3)$$

where $g(T)$ again represents an arbitrary function of the temperature.

The relations (1), (2) and (3) are, of course, also applicable to the special case of the black body radiation. In this application, however, a peculiarity characterizing the black body radiation is of decisive importance. As is well known,³ it follows at once from Kirchhoff's radiation law that in the case of radiation, the ratio of the internal energy to the volume is simply a function of the temperature, or

$$U(V, T) = V \cdot u(T). \quad (4)$$

Since the last term in (3) is independent of the volume, we have to put in (3), if it is applied to the special case of the black body radiation,

$$g(T) = 0. \quad (5)$$

If therefore p be given as a function of the energy density u by Maxwell's relation

$$p = u/3, \quad (6)$$

the insertion into (3) of the value of p as given by (6) leads, indeed, to a unique determination of the energy density as a function of the temperature, if we consider equations (4) and (5); we obtain Stefan's law in the form

$$u = a T^4, \quad (7)$$

a being an integration constant. In the usual presentations (5) is tacitly assumed, without any mention of the fact that (5) is a consequence of (4).

In reversing Boltzmann's well-known derivation of Stefan's law we may, however, also assume (7) to be given, the factor a being quite a definite constant. The combination of (2) and (7) then leads at once to the relation

$$p = \frac{1}{3} a T^4 + T f(V). \quad (8)$$

On the basis of this relation (7), Stefan's law, appears compatible with (6), Maxwell's formula, only if

$$f(V) = 0. \quad (9)$$

We cannot, therefore, deduce Maxwell's formula from Stefan's law by means of the first and second laws unless we can show that $f(V)$ must vanish.

Classical thermodynamics as resulting from the first and second laws does not suffice for this purpose. It proves to be necessary to take Nernst's heat theorem also into account, in Planck's form, according to which the entropy of a system must vanish in the limit for the absolute zero, or

$$\lim_{T \rightarrow 0} S = 0. \quad (10)$$

The differential of the entropy is determined by the first and second laws, in accordance with the well-known relation

$$dS = (dU + p dV)/T. \quad (11)$$

In this equation we may insert for p the value from (8), and according to (4) and (7) we may put

$$U = a V T^4. \quad (12)$$

On integrating we then find

$$S = \frac{4}{3} a V T^3 + \int f(V) dV + C \quad (13)$$

where C denotes an arbitrary quantity which, however, is independent of the volume.

Quite independently of Nernst's heat theorem, we may prove that the quantity C must vanish. For, according to (4) U must vanish for vanishing V ; but, if C should differ from zero, then, according to (13), the entropy would also differ from zero for this case. This, however, is inconceivable since we cannot ascribe an entropy differing from zero to a system whose volume and internal energy vanish and which, therefore, is no system at all.⁴ Hence, the last term in (13) must be zero, or

$$C = 0. \quad (14)$$

If, however, Nernst's heat theorem is to hold in Planck's form, $f(V)$ must also vanish. Thus (9) appears to be proved and the derivation from Stefan's law of Maxwell's formula for the radiation pressure appears to be complete.

Finally, we shall assume both Stefan's law and Maxwell's formula for the radiation pressure to be given, in combination with classical thermodynamics. Then a comparison of (6) and (8) first shows the necessity of the vanishing of $f(V)$. Furthermore, as was shown before, (14) may be gained without using Nernst's heat theorem at all. Therefore it appears to be proved on the basis of Stefan's law and the electro-magnetic formula for the radiation pressure that (10) must be fulfilled for the radiation. Thus in the special case of the black body radiation Nernst's heat theorem in Planck's formulation⁵ may be deduced simply from classical thermodynamical and electrodynamical principles.

¹ A more general treatment including applications to the thermodynamics of long-chain-molecules will be given in a forthcoming paper of one of the authors (E. G.) whereas a forthcoming paper of the other author (A. E. H.) will deal with the interpretation of the thermodynamics of the black body radiation from the viewpoint of the photon theory.

² Cf. e.g., Mark W. Zemansky, *Heat and Thermodynamics*, p. 233 (1937).

³ Cf. e.g., M. Planck, *Theory of Heat*, p. 193 (1932).

⁴ Cf. e.g., P. S. Epstein, *Textbook of Thermodynamics*, p. 333 (1937).

⁵ In Nernst's own original formulation his heat theorem simply postulated that the entropy must remain finite in the limit for $T \rightarrow 0$. This postulate is fulfilled if the specific heat at constant volume, that is

$$C_V = (\partial U / \partial T)_V$$

vanishes for $T = 0$. This consequence follows at once from Stefan's law since the differentiation of (6) with respect to the temperature yields the relation

$$C_V = 4aVT^3.$$

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

June 15, 1938

Number 6

Copyright 1938 by The National Academy of Sciences

EVIDENCE FOR A SECOND THIAMIN

BY WILLIAM J. ROBBINS AND FREDERICK KAVANAGH

THE NEW YORK BOTANICAL GARDEN

Communicated May 10, 1938

In an earlier paper¹ the authors reported briefly the effect on the growth of *Phycomyces Blakesleeanus* of each of 36 pyrimidines when used in a nutrient solution with 4-methyl-5 β hydroxyethyl thiazole, one of the two intermediates from which Williams and Cline synthesized thiamin (vitamin B₁). Thirty of these compounds had been tested by the writers and six of them by other investigators. At that time we were unable to determine the significance of the CH₃ group in the second position on the pyrimidine ring because suitable compounds were not available.

Through the courtesy of the I. G. Farbenindustrie Aktiengesellschaft, samples of 5-bromo-methyl-6-amino pyrimidine and 2-ethyl-5-bromo-methyl-6-amino pyrimidine were secured. The first of these compounds differs from the pyrimidine used by Williams and Cline in having hydrogen substituted for the methyl group in the second position; the second compound has the ethyl group for the methyl group.

When ten units of 5-bromo-methyl-6-amino pyrimidine were used with ten units of the vitamin thiazole in the nutrient solution previously described¹ no growth of *Phycomyces* occurred. The second compound used in the same way was as effective as the 2-methyl-5-bromo-methyl-6-amino pyrimidine. For both sets of experiments the solutions containing the supplements were sterilized by heating at 15 lb. pressure for 15 minutes.

We conclude that for *Phycomyces* the methyl group in the second position on the vitamin pyrimidine is important since the substitution of hydrogen for the methyl group eliminated the activity of the pyrimidine. However, the effectiveness of the compound is largely or entirely retained when the ethyl group replaces the methyl group in the second position.

This observation suggests that there may be more than one compound capable of functioning in the living organism as thiamin does; a methyl thiamin, the compound originally isolated and synthesized, and an ethyl thiamin differing from the former in having the CH₃ group in the second position on the pyrimidine ring replaced by the C₂H₅ radical.

In our experiments we have used the pyrimidine and thiazole compounds as such. From evidence presented previously¹ we believe, however, that the vitamin molecule (not the intermediates themselves) functions in facilitating the growth of *Phycomyces*. When a mixture of a particular pyrimidine and a particular thiazole is effective we conclude that the vitamin molecule or a substitute therefor is synthesized by the organism from the intermediates. It is on this basis that we suggest the existence of an ethyl thiamin.

There is of course the possibility that the fungus was able to substitute the methyl for the ethyl group and that the final effective product was identical with thiamin. We have no evidence on this point, though we are inclined to assume that this is not probable.

If there is an ethyl thiamin as we suggest there may also be other thiamins such as propyl and butyl. The investigation of this possibility must necessarily wait on the availability of suitable pyrimidines.

¹ William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, 24, 141-145 (1938).

² William J. Robbins and Frederick Kavanagh, *Ibid.*, 23, 499-502 (1937).

AN ASTACENE-LIKE CAROTENOID FROM A PACIFIC COAST ANEMONE, *EPIACTIS PROLIFERA*

BY DENIS L. FOX AND CHESNEY R. MOE

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA,* AND SAN DIEGO STATE COLLEGE

Communicated May 14, 1938

While many fishes, mollusks, crustaceans, anemones and members of other phyla contain known carotenoids of recognized plant origin, including any or several carotenes and xanthophylls, contributions from various investigators during recent years have brought out the fact that numerous marine animals contain also previously undescribed carotenoids as well, resulting in a steady extension of our list of known lipochromes peculiar to animals. These animal lipochromes fall chiefly into the classes of acidic and xanthophyllic carotenoids. (See reference in Zechmeister, 1934, 1937; Lederer, 1933, 1935, 1938; Heilbron, Jackson and Jones, 1935.)

Heilbron and his co-workers (op. cit.) have given some attention to lipochromes of several species of sea anemones, and report, among other pigments, including new animal xanthophylls, a deep red, acidic carotenoid ester from *Actinoloba dianthus*, which, when hydrolyzed and crystallized in pure condition, showed a single absorption maximum in CS₂ at 495 mμ.

From *Tealia felina* they obtained, along with other carotenoids, a different orange-red acid which showed a single absorption maximum in the same solvent of 500 $m\mu$, whether free or in the natural esterified condition. While both of these pigments resembled astacene in their general color, acidic character and the possession of a single absorption band, neither was identical with it, since the positions of the respective bands were somewhat removed toward the violet from that of astacene, which is reported by Lederer (1935) to have an absorption maximum at 510 $m\mu$ in CS_2 ; Karrer and Solmssen (1935) report 514 $m\mu$ (497 $m\mu$ before hydrolysis) as the maximum of astacene in CS_2 ; they obtained the pigment from the sponges *Axinella crista-galli* and *Suberites domuncula*, as well as from a gastropod mollusk *Pleurobranchus elegans*, and a sea-star *Echinaster sepositus*. Additional critical data which set Heilbron's red carotenoids apart from astacene were in the melting points: while astacene melts at 240–243°C. (Zechmeister, 1934; Lederer, 1935), Heilbron's astacene-like carotenoid acids melted at 195–196°C. and 205–208°C., respectively.

Astacene is a tetraketo-beta-carotene (Karrer and Loewe, 1934). In view of the exceptional change brought about by various animals in an otherwise intact carotene or xanthophyllic molecule, resulting in the presence of four ketone groups (two on either terminal ionone ring) it would seem not unlikely that other typically animal carotenoids of similar but not identical physical and chemical properties might be synthesized by members of certain phyla. Of this we have already considerable evidence, as brought out above; facts reported below would appear to add information of similar significance.

An important question regarding the unique oxidative changes, such as are involved in the formation of astacene in animals is: Do those lipochromes which are peculiar only to animals result from biochemical differences in various animal species, all of which may modify a *common* carotenoid contained in their diet, or may the various animal species instead use each a *different* carotenoid as substrate for the synthesis? As an instance, we may consider for a moment the fact that taraxanthin, which seems to occur commonly in numerous marine forms (Fox, 1936; Young and Fox, 1936; see also citations in Zechmeister, 1937) has the empirical formula $C_{40}H_{56}O_4$ (Kuhn and Lederer, 1931), while the empirical formula for astacene is $C_{40}H_{48}O_4$ —a difference of 8 H atoms, corresponding to the conversion of four secondary alcoholic groups to four ketonic groups. This particular picture is offered not as a suggestion of the exact compounds involved, but as a general working hypothesis for possible use as a guide in future metabolic studies to be made in pursuit of the problem.

This contribution concerns an interesting anemone, *Epiactis prolifera*, Verrill, found along the California coast from Puget Sound to San Diego (Johnson and Snook, 1927). Its young complete their development in

numerous circular pits around the outside of the parent's body, migrating later to positions on the substrate in the immediate vicinity. The color of this animal is red, orange-red or occasionally red-brown. It is of relatively small size, adults having a pedal disc diameter of 10-20 mm., and a similar height.

During June and July, 1936, great numbers of this species bearing young were taken from the kelp beds off the coast at San Diego, where they were found attached to the blades and stipes of the giant kelp *Macrocystis pyrifera*, along with other small animals, including species of the sedentary, tube-building serpulid worm *Spirorbis* and the small amphipod *Caprella*. We were led to believe that the anemone fed, at least in part, upon the latter amphipod.

When a few of the whole animals were ground in a mortar with sand and ethyl alcohol, the tissues yielded a yellow-orange extract. The epiphasic character of the pigment at this stage was demonstrated by its quantitative extraction from >90% alcohol by shaking with ligroin. Hydrolysis was brought about by treatment with alcoholic KOH for two or three hours in a hot water bath. Subsequently all pigment remained dissolved in the aqueous alcohol layer. Attempted extraction with ligroin now resulted in little or no yield of pigment to the latter solvent, even after diluting the alcohol layer with much water. Upon adding concentrated sodium chloride solution, the emulsion broke, the pigment being thrown out at the interface of the two liquids as a red potassium salt. Slight acidification of the system with acetic acid immediately hydrolyzed the potassium salt, and the free pigment was now dissolved in the ligroin layer. The neutral pigment was subjected to partition between 90% CH₃OH and ligroin; part of it migrated into the alcoholic layer below; on adding a drop of concentrated alkali all pigment was transferred from the ligroin phase into the alcoholic layer; re-acidification returned the pigment mostly into the ligroin phase as before.

Many more of the animals, brought in on the kelp, on July 13, 1936, were carefully removed and placed in glass tanks of running sea water. After twenty-four hours, during which interval no deaths occurred, and the anemones attached themselves to the walls of the container, about fifty animals were detached, rolled carefully and cleaned on filter paper to remove water and possible extraneous sources of pigment. The net weight of the mass of animals so treated was 13 grams. Upon dropping into pure methyl alcohol, much protein, probably of a mucous nature, was soon precipitated. The bodies of the anemones changed in color from the original orange or orange-pink to coral pink, while the alcohol took on an orange-yellow color. After storing overnight, under an inert atmosphere of illuminating gas, in a glass-stoppered bottle placed in an electric refrigerator, the methyl alcohol was decanted from the anemones, fresh 95% ethyl alcohol was added and

the tissues reduced to very fine particles by grinding for one and one-half hours in an Abbé ball mill. The mass was next extracted of all remaining pigment by washing with successive applications of alcohol, drawing the solvent off through a sintered glass filter. Finally the combined methyl- and ethyl-alcoholic extracts were treated in a closed system with alcoholic KOH in a hot water bath for two hours. The hydrolysate was stored under illuminating gas in the refrigerator overnight.

Dilution of the hydrolysate and a single extraction with ligroin yielded a very small amount of pigment to the latter solvent; subsequent washes yielded no more, all pigment remaining, as in the earlier experiment, in the aqueous alcoholic layer below, even after considerable dilution.

The trace of pigment extracted in ligroin from the diluted alkaline alcoholic hydrolysate was separated, carefully washed free of alcohol, freed of traces of water and passed through a micro-chromatographic column of dry CaCO_3 ; no pigment was adsorbed. The ligroin in the filtrate was evaporated, and the residual pigment taken up in CS_2 , giving an orange-colored solution. This pigment was present in too small quantities to obtain good spectrophotometric measurements, but the facts that, after prolonged treatment with alcoholic KOH, it remained quantitatively epiphasic in the partition test between ligroin and 90% CH_3OH , and was unadsorbed by CaCO_3 , marked it as a carotene. The main mass of pigment was now subjected to the same treatment as described in the first investigative procedure, and the earlier observations were duplicated; acidification freed the red carotenoid acid from combination as a potassium salt and rendered it extractable with ligroin; ligroin solutions of the pigment were orange-yellow; solid pigment residues were magenta-red in color, as were CS_2 solutions; partition tests of the free pigment between ligroin and 90% CH_3OH showed its solubility in both layers; alkalization of the system brought the pigment entirely back into the alcohol layer.

Chromatographic treatment of a ligroin solution of the free pigment upon a column of CaCO_3 showed a single slowly advancing rose-red zone. This procedure was repeated and gave an identical observation. The pigment was gradually washed through the column with added quantities of ligroin; when the solvent finally came through the column colorless, the filtered pigment solution was removed from the receiving flask and kept separately. Additional ligroin containing a little CH_3OH now desorbed a trace of pigment which had remained in the column. This trace was too small to attempt to classify it further. Its behavior suggests that it might have been a xanthophyll, present originally as an ester.

The absorption spectra of the free carotenoid acid and its ester, each in carbon disulphide, were determined separately by the direct visual method of matching light intensities through a Bausch and Lomb spectrophotometer. Figure 1 shows arbitrary units of relative intensity ($d = \log I_0/I$, where I_0

is the intensity of the light beam passing through the solvent alone, and I the intensity of the light beam passing through the solution of the pigment) plotted against λ , the wave-length in Å units. The lower curve of the ester and the upper curve of the free acid are not superposed, since there was no attempt to use identical concentrations (molecular weights being unknown) but merely to determine the region of the absorption maxima at any convenient concentration. It is seen that the pigment, whether free or esterified, shows a single broad band at 5000 Å with a high degree of symmetry in the slopes for a length of 500 Å on either side. (Compare asymmetry

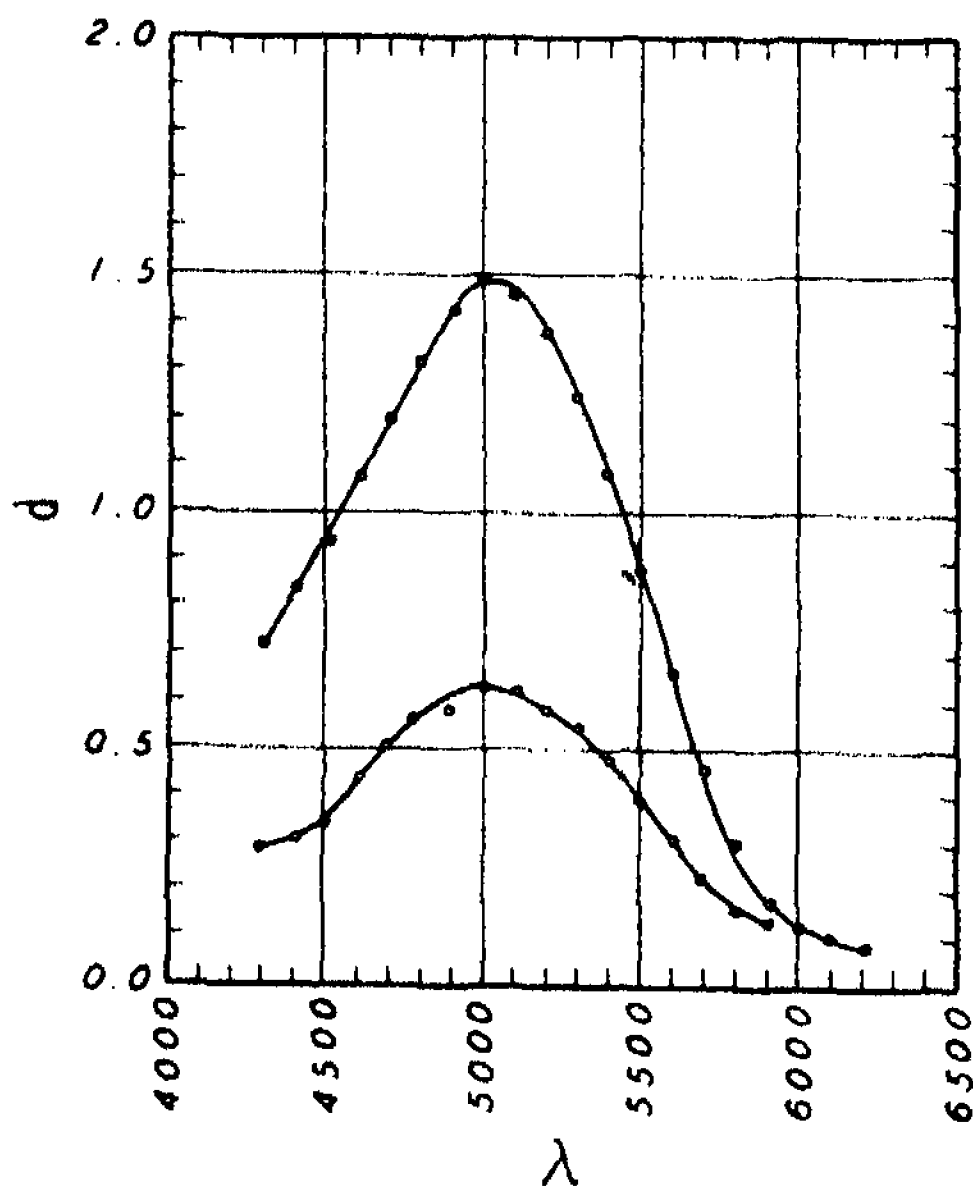


FIGURE 1

Absorption maximum of the chief carotenoid from *Epiactis prolifera*; upper curve, free acid; lower curve, natural ester. Ordinates: $d = \log I_0/I$. Abcissae: wave-length, λ , in Å.

the pigment more similar to certain other acidic carotenoids found in other species of anemones by Heilbron, *et al.*

The increasing number of known carotenoids peculiar to the animal kingdom emphasizes the question as to whether the biochemical differences in animal species are in the mode of action upon a single plant carotenoid, or in selective action of different animal species upon different plant carotenoids.

Acknowledgments.—We are grateful to Mr. M. J. Walsh, Manager of the Kelp Laboratories, San Diego, California, and to Mr. L. Criswell, the skip-

of astacene curve in pyridine, which has a considerably steeper slope between 5000 Å and 5500 Å than between 5000 Å and 4500 Å; illustrated in Kuhn and Lederer, 1933; Zechmeister, 1934; Lederer, 1935.)

Summary.—The small red-orange anemone, *Epiactis prolifera*, Verrill, which inhabits the ocean waters of the Pacific Coast, owes its striking color to considerable quantities of a red acidic carotenoid, present entirely in esterified form. The animal contains also a trace of one of the carotenes, and perhaps traces of an esterified xanthophyllic carotenoid. The character of the acidic carotenoid does not identify it with astacene, but its absorption spectrum renders

per of Mr. Walsh's boat, for bringing to us the specimens of *Epiactis*. We are also indebted to various members of the local project of the Federal Works Progress Administration for aid in the preparation of the manuscript.

Contributions from the Scripps Institution of Oceanography. New Series No. 17.

Fox, D. L., "Further Studies of the Carotenoids of Two Pacific Marine Fishes, *Fundulus parvipinnis* and *Hypsypops rubicunda*, and of a Marine Annelid, *Thoracophelia* sp.," *Proc. Nat. Acad. Sci.*, **22**, 50-54 (1936).

Heilbron, I. M., Jackson, H., and Jones, R. N., "The Lipochromes of Sea Anemones, I. Carotenoid Pigments of *Actinia equina*, *Anemonia sulcata*, *Actinoloba dianthus* and *Tealia felina*," *Biochem. Jour.*, **29**, 1384-1388 (1935).

Johnson, M. E., and Snook, H. J., *Seashore Animals of the Pacific Coast*, Macmillan Co., New York (1927).

Karrer, P., and Loewe, L., "Constitution of Astacin," *Nature*, **133**, 936 (1934).

Karrer, P., and Solmssen, U., "Occurrence of Carotenoids in Some Marine Animals," *Helv. Chim. Acta*, **18**, 915-921 (1935).

Kuhn, R., and Lederer, E., "Taraxanthin, ein neues Xanthophyll mit vier Sauerstoffatomen," *Zeit. physiol. Chem.*, **200**, 108 (1931). (Cited by Zechmeister, 1934.)

Kuhn, R., and Lederer, E., "The Pigments of Lobster (*Astacus gammarus* L.) and Their Parent Substance, Astacin," *Ber.*, **66B**, 488-495 (1933).

Lederer, E., "Note sur les Lipochromes d'*Actinia Equina* L.," *Compt. rend. Soc. biol.*, **113**, 1391-1392 (1933).

Lederer, E., *Les Carotenoides des Animaux*, Paris, Hermann et Cie (1935).

Lederer, E., "Recherches sur les Carotenoides des Animaux Inférieurs et des Cryptogames." Thesis, University of Paris, Lons-le-Saunier Imprimerie et Lithographie Maurice Declume (1938).

Young, R. T., and Fox, D. L., "The Structure and Function of the Gut in Surf Perches (Embiotocidae) with Reference to Their Carotenoid Metabolism," *Biol. Bull.*, **71**, 217-237 (1936).

Zechmeister, L., *Carotinoide*, Berlin, Julius Springer (1934).

Zechmeister, L., "Die Carotinoide in tierischen Stoffwechsel," *Ergebn. der Physiol., biol. Chem., und exp. Pharmacol.*, **39**, 117-191 (1937).

THE STRUCTURE OF THE RING-GLAND (CORPUS ALLATUM)
IN NORMAL AND LETHAL LARVAE OF *DROSOPHILA*
MELANOGASTER

BY BERTA SCHARRER AND ERNST HADORN

DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO, AND DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF ROCHESTER

Communicated April 29, 1938

In a recent paper (Hadorn, 1937a) it was shown that puparium formation in *Drosophila melanogaster* is brought about by a hormone. As the place of origin of this pupation hormone, an endocrine gland was described lying in the neighborhood of the brain. As long as no homology to other organs of that type in insects was found, this gland was called "ring-gland," as it is identical with the "Ring" of Weismann who in 1864 described it as a supporting structure for the dorsal blood vessel of *Calliphora vomitoria*.

Experiments with this ring-gland had the following results:

(1) Compared with normal larvae the puparium in the *Drosophila* mutation "lethal-giant" (*lgl*-) is formed later or not at all. This process can be accelerated by implantation of a mature normal ring-gland to *lgl*-larvae, but not by any other tissue (for instance brain) from genetically normal larvae.

(2) If ring-glands from normal larvae ready for pupation are implanted into younger normal larvae those will show a premature puparium formation. The degree of acceleration depends upon the time of injection and also upon the number of glands injected, three being more effective than one. The same accelerative effect can be obtained with mature normal ring-glands of *Drosophila melanogaster* in larvae of the more slowly developing *Drosophila hydei* and in lethal male crosses of the combination *Drosophila melanogaster* × *Drosophila simulans* (Hadorn, 1937b, Hadorn and Neel, 1938).

In the present paper the histological structure of the ring-gland was studied, the following questions being of particular interest:

(1) Is there histological evidence for the secretory activity of the ring-gland cells?

(2) Do ring-glands of old and young normal larvae (temporarily or permanently) show a different appearance indicating perhaps a functional cycle?

(3) Can normal ring-glands be differentiated from lethal ones?

(4) Is the ring-gland of *Drosophila* homologous with the corpora allata found in other insects?

1. *Material*.—As seen in table 1, normal and lethal larvae (*lgl*-) of *Drosophila melanogaster* were preserved at different ages in Bouin, Zenker-formol

or Champy. The material was embedded in celloidin, paraffin sections being used only as control material. The 5 μ serial sections (Rubaschkin method) were stained with Heidenhain's iron hematoxylin, Mallory-azan or van Gieson.

TABLE 1

AGE OF LARVA	GENETIC CONSTITUTION OF MATERIAL	NUMBER OF INDIVIDUALS INVESTIGATED
1 day	normal + <i>lgl</i> -larvae together	5
1 day + 19 hours	normal + <i>lgl</i> -larvae together	4
2 days + 22 hours	normal larvae	4
	<i>lgl</i> -larvae	4
3 days	normal + <i>lgl</i> -larvae together	3
3 days + 16 hours	normal larvae	4
	<i>lgl</i> -larvae	3
3 days + 22 hours	normal larvae	6
	<i>lgl</i> -larvae	2
4 days + 4 hours	normal larvae	4
	<i>lgl</i> -larvae	1
4 days + 16 hours	normal larvae	4
	<i>lgl</i> -larvae	1
4 days + 18 hours	normal larvae	4
	<i>lgl</i> -larvae	1
4 days + 22 hours	<i>lgl</i> -larvae	6
5 days + 5 hours	<i>lgl</i> -larvae	5
5 days + 20 hours	<i>lgl</i> -larvae	2
6 days	<i>lgl</i> -larvae	4

2. *Appearance of the Ring-Gland of a Normal Larva Ready for Pupation.*—As shown before in a diagram (Hadorn, 1937a, p. 480, Fig. 2), the ring-gland is located dorsally between the two hemispheres of the cerebral ganglion. Its longitudinal diameter is 200 μ , the largest transverse extension 130 μ and the depth 30 to 40 μ . As described in other Diptera by former investigators (Giacomini, 1900, Snodgrass, 1924), the "ring" is anchored in its position by tendinous structures. Tracheae penetrate and pass through the ring-gland; the cells surrounding them (imaginal bud cells, Burtt, 1937) can easily be differentiated from the gland cells. Two longitudinal tracheae arising from the cerebral hemispheres take their way forward and outward within the ventral part of the ring-gland. As

in *Calliphora* (Burt, 1937), these two tracheal stems are connected by a transverse trachea. Significant is the fact that the ring encircles the aorta, the main part of the gland tissue being closely attached to the dorsal part of the vessel, whereas ventralward the ring becomes much thinner and ends more or less incompletely. The aorta itself can be traced from the heart to its termination in front of the ring-gland. The cells forming the thin wall of the vessel are thickened at those points where the oval-shaped nuclei are located. Sometimes one can observe in suitably stained sections that these cells contain a few delicate myofibrils showing a transverse striation; but only the part of the aorta posterior to the ring seems to be contractile. According to Giacomini (1900) the anterior portion of the dorsal vessel is incomplete in *Eristalis tenax*, consisting only of the upper half, whereas the lower lip ends immediately in front of the ring. The same is true in *Drosophila*; here this dorsal portion of the vessel is exceedingly thin, containing only comparatively large nuclei and ending in a membranous structure. Sometimes blood cells are found in the vessel especially in its frontal enlarged region.

To establish the relationship between ring-gland and stomatogastric nervous system is more difficult in *Drosophila* larvae than in other less tiny Diptera. One can trace the recurrent nerve passing forward and forming a small ganglion (evidently homologous to the median ganglion of Lowne, 1890-95, and Burt, 1937), which is in contact with the antero-ventral portion of the ring.

The cells forming the ring-gland are of two different types which can be differentiated by their size as well as by their position. The main part of the ring is built by large cell elements (about 15-40 μ in diameter) which are very characteristic. Their cytoplasm is deeply staining and shows a more or less granular structure. In some of the cells vacuoles of different size and irregular form can be found. The cell boundaries are easily distinguished in most of the preparations. The large nuclei of those elements are round or oval and have diameters from 10 to 17 μ ; they contain one or two nucleoli (about 3 μ in diameter). The chromatin is arranged in more or less distinct chromosome-like bodies as, for instance, in *Chironomus* (Burt, 1937). Thus these cells are similar in their general appearance to the elements of the salivary glands.

The cells of the smaller type form the central part of the ring-gland surrounding the anterior region of the ring-hole, and thus lying in the direct neighborhood of the dorsal vessel. Especially in its anterior part, this cell group shows a very intimate contact with the thin dorsal lip of the aorta. The area occupied by these smaller cells is about 60 μ long (from the ring-hole oralward in the anterior direction), 30 μ wide and 30-40 μ deep. In some preparations (azan) a very delicate connective tissue membrane can be observed surrounding this cell group as well as the whole ring. In a full

grown ring-gland of a mature larva one may count about 20 cells of that type. The smaller cells of the ring are in their general aspect similar to its larger elements. Their nuclei are round ($5\text{--}6\ \mu$ in diameter) and have one nucleolus. As in the large ring cells, the nucleolar membrane is distinct. The cell diameters are from about 8 to $20\ \mu$. The cytoplasm, being less vacuolated, stains somewhat deeper than that of the large cells. Nerve cells as described by Burt (1937) in *Calliphora vomitoria* have not been found in the ring-gland of *Drosophila*. No direct histological evidence could be brought forward until now for a glandular activity of the ring-gland cells (i.e., presence of granules or colloid substances in the cytoplasm, etc.). But the general appearance of those cells (affinity of the cytoplasm to stains, vacuolization, large size of nuclei and their large amount of chromatin) do at least not speak against a secretory activity.

3. *Homology of Ring-Gland and Corpora Allata*.—Already in the first paper on *Drosophila* (Hadorn, 1937a) the question of a homology between ring-gland and corpora allata has been discussed. At the same time Burt (1937) working with *Calliphora vomitoria* concluded that Weismann's ring represents a modified corpus allatum. He did not, however, prove this assumption by an embryological study, but he gave a comparative survey emphasizing the great variability in shape, size and position of the corpora allata not only within the insects but even within the Diptera. As yet the embryological development of the ring-gland in *Drosophila* has not been studied, this small species not being the most favorable object among the dipterous insects. But on the basis of a histological study of different postembryonic stages we came to the same result in *Drosophila* as Burt in *Calliphora*. Among further morphological correspondences between ring-gland and corpora allata, we may mention their position in the body and relationship to other organs (especially to the dorsal vessel and to the tracheal system) and the histological properties of their elements (affinity to plasma stains, granular cytoplasm containing vacuoles, appearance of the nuclei, etc.).

There is one point more to be discussed concerning the two different cell types in the ring-gland of *Drosophila*. As mentioned above, the smaller cell elements have a well circumscribed position within the ring, being even separated from the large gland-cells by a fine membrane. Therefore the question arises whether this well defined central cell group forms a special part of the corpus allatum or must be separated from it as a different structure. It is very probable that the small ring-cells represent the corpora cardiaca (Pflugfelder, 1936–37, p. 47; esophageal ganglia, Burt, 1937; pharyngeal ganglia of other investigators). Several reasons speak in favor of such an assumption:

(1) The name of corpora cardiaca was proposed by Pflugfelder for the "pharyngeal ganglia" of other authors, because of their intimate re-

lationship to the blood vessel system; in some insects (for instance in Nabis, Rhynchota) the corpora cardiaca represent even a modified and thickened wall of the heart or aorta, respectively. In *Drosophila* the close contact between the small ring-cells and the thin membrane of the dorsal vessel is obvious.

(2) Another reason for discarding the former name of pharyngeal or esophageal ganglia is that, at least in part of the insects, their purely ganglionic nature can no longer be maintained. Pflugfelder (1937a and b) showed that for instance in *Dixippus morosus* part of the cells of the corpora cardiaca contain (secretory or excretory?) osmio- and fuchsinophile substances. The small cells of the *Drosophila* ring have a glandular appearance rather than a nervous.

(3) The position of the corpora cardiaca in a number of insects (for instance in *Syromastes*, Rhynchota) is closely oralward of the corpora allata, with only a thin membrane separating these structures from each other. One may easily assume that in *Drosophila* the corpora allata on the basis of their special development have grown around their corpus cardiacum, thus building an even more intimate connection. The thin membrane found between the smaller and larger ring cells has already been mentioned.

(4) From other insects we know that the cell elements of the corpora cardiaca are smaller than those of the corpora allata.

(5) There has not been found as yet another organ in cyclorrhaphous insects which could be homologized with the corpora cardiaca.

In this connection we may ask what conditions we find in the other cyclorrhaphous insects. In the ring of *Lucilia* (we have studied only two specimens to date) we found also two cell types of different size and appearance, but they are not so separated from each other as in *Drosophila*. According to Burt (1937, p. 21, Fig. 6) the same seems to be true for *Calliphora vomitoria*. Whether these smaller cells scattered in the ring-glands of *Lucilia* and *Calliphora* correspond to the group of small elements in the *Drosophila* ring cannot be decided at the present. In the same way the question of a homology between the small ring-gland cells and the corpora cardiaca will remain uncertain as long as we do not have a more detailed study of those organs in different groups of insects.

4. *Development of the Ring-Gland in Normal and Lethal Drosophila Larvae.*—In young larvae (age: 1 day, 1 day and 19 hours) the ring-gland elements (diameter of nuclei 3μ) cannot yet be differentiated with certainty from the surrounding tissues, especially from the salivary glands. Normal larvae aged 2 days and 22 hours show already a typical ring. The ring-gland cells in this stage as well as in 3 day old larvae are to be distinguished from those of older ones by their size, the diameters of the nuclei being only from 6 to 8μ . The smaller cells are already present in the center of the ring, but they do not differ so much in size from the large ones as later. In

three day old larvae the diameters of the small cell nuclei are from 4 to 5 μ . At the age of 3 days and 16 hours the ring-glands as well as their elements have reached their full size (Fig. 1) and now do not differ from the oldest normal stages described above.

Accordingly we may state that the growth of the ring-gland during the larval development of *Drosophila* takes place by an increase in the size of the cells, but not by a considerable increase in their number. That is why mitoses are to be found only exceedingly rarely in the ring-gland cells.

Also in the *lgl*-larvae the ring-gland has reached its final size at the age of 3 days and 16 hours but there is one striking difference in contrast to normal larvae. The ring-glands of *lgl*-larvae are considerably smaller than the normal. Even in 5-6 day old *lgl*-larvae (Fig. 2) the length of the ring is only about 120 μ , the width 70 μ and the depth 30 μ . As we may expect from what was said about the growth of the normal ring, the comparative smallness of the lethal ring-gland is due to the fact that the cells are smaller than normal. The nuclei of these large ring cells do not reach greater diameters than from 7 to 10 μ . In this connection it must be emphasized that an *lgl*-larva is as a whole not smaller than a normal one of the same age. Besides the size there have been observed no other marked differences between normal and lethal ring-gland cells, at least not with the technique used and on the basis of the present material. The small cell type in a lethal ring-gland does not differ in size from that in normal ones, neither are those cells less in number.

From the experiments of Hadorn (1937a) it was concluded that an *lgl*-

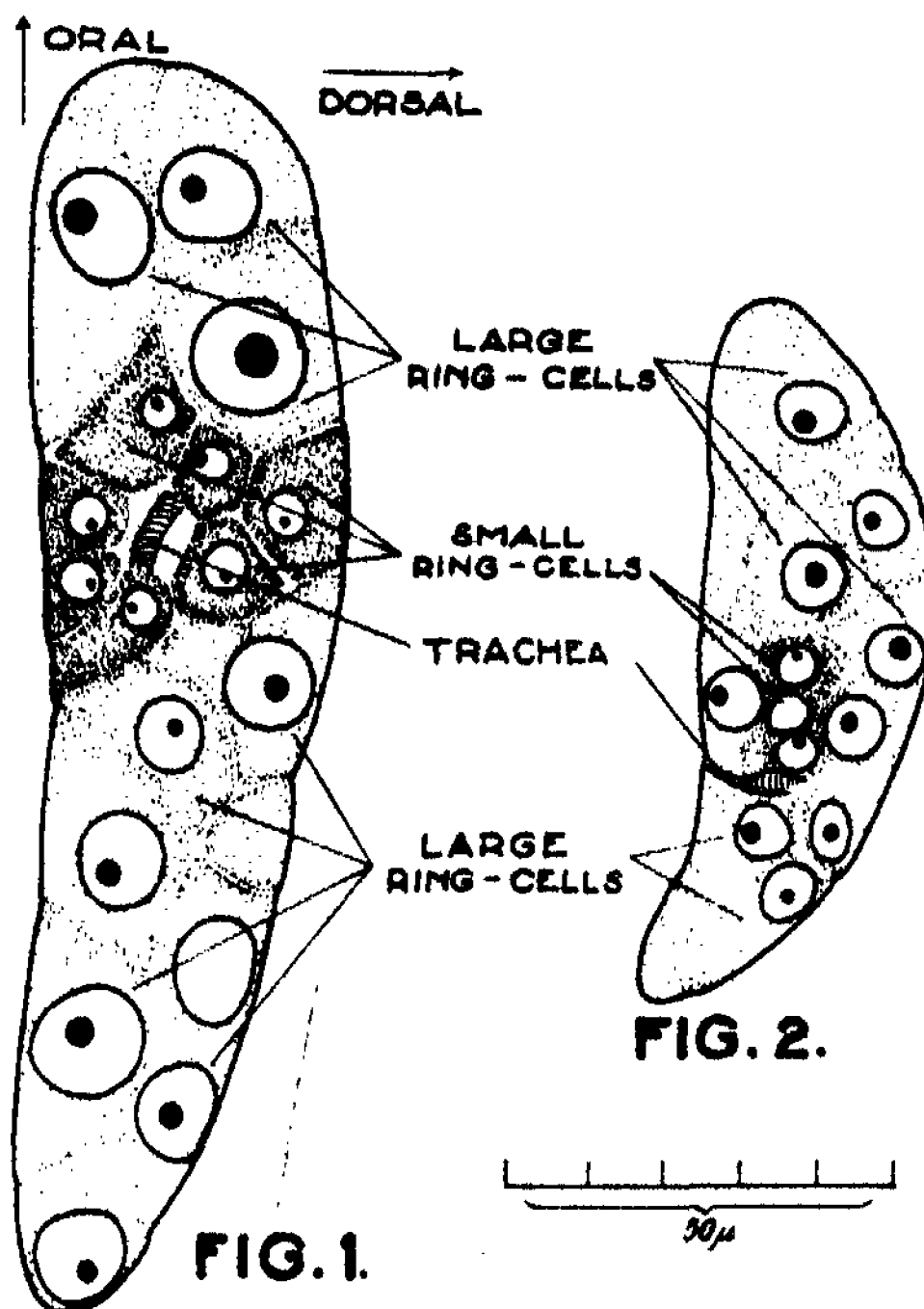


FIGURE 1
Paramedian sagittal section of ring-gland (corpus allatum) of a normal *Drosophila* larva (age 3 days and 16 hours). Camera lucida drawing.

FIGURE 2
Corresponding section of a lethal (*lgl*-) larva (age 5 days and 5 hours).

ring-gland does not furnish enough hormone to bring about puparium formation at the right time. The histological statements reported above may explain this fact. An *lgl*-ring-gland never grows to full size and apparently does not as a rule reach the hormone producing phase. It remains rather at a stage typical for a young normal larva of about 3 to 3½ days. Unpublished transplantation experiments of Hadorn and Neel show that it is impossible to promote puparium formation with unripe glands even if they are of genetically normal constitution.

We are, however, not allowed to assume that an *lgl*-gland does not produce any hormone at all, since many of the lethal larvae later may form puparia. It is rather possible that an *lgl*-ring-gland, in spite of its underdeveloped histological structure, furnishes a small amount of hormone which reaches the necessary threshold only with great retardation.

A more detailed discussion of the literature will be given in another paper.

5. *Summary.*—The finer structure of the ring-gland (Weismann's "Ring") in *Drosophila melanogaster* is described. For the secretory activity of the ring-gland cells (pupation hormone) as suggested by Hadorn (1937) no histological evidence could be established. The ring-gland, or at least its greater part (large cells), is homologous to the corpora allata found in other insects. The question of a homology between the small ring-cells and the corpora cardiaca (Pflugfelder) is discussed. During the larval development of *Drosophila* the growth of the ring-gland (corpus allatum) takes place by an increase in the size of the cells. In the grown-up *lgl*-larvae the ring-gland as well as its cell elements is considerably smaller than normal. It is therefore concluded that the retardation in the puparium formation of the *lgl*-larvae is caused by an underdevelopment of their corpora allata.

This research was aided by a grant to the University of Chicago by the Rockefeller Foundation.

¹ Burt, E. T., *Proc. Roy. Soc. London*, **B124**, 13-23 (1937).

² Giacomini, E., *Ann. Fac. Med.-Chir. Perugia*, **12**, 131-217 (1900; quoted from Snodgrass 1924).

³ Hadorn, E., *Proc. Nat. Acad. Sci.*, **23**, 478-484 (1937a).

⁴ Hadorn, E., *Naturwiss.*, **25**, 681-682 (1937b).

⁵ Hadorn, E., and Neel, J., *Genetics*, **23**, 151 (1938).

⁶ Lowne, B. T., *The Blow Fly*, London (1890-1895).

⁷ Pflugfelder, O., *Zoologica* (ed. by R. Hesse) **34**, H. 93, 1-102 (1936-37).

⁸ Pflugfelder, O., *Verhandl. Deutsch. Zool. Ges.*, Bremen (1937); *Zool. Anzeiger*, **10**, Suppl., 121 (1937a).

⁹ Pflugfelder, O., *Z. wiss. Zool.*, **149**, 477-512 (1937b).

¹⁰ Snodgrass, R. E., *Jour. Agric. Res.*, **28**, 1-36 (1924).

¹¹ Weismann, A., *Z. wiss. Zool.*, **14**, 187-336 (1864).

ON NORMAL DIVISION ALGEBRAS OF INDEX 5

BY RICHARD BRAUER

UNIVERSITY OF TORONTO

Communicated May 10, 1938

1. Let D be a normal division algebra of index $m = 5$ (i.e., of order 25) over a field F .¹ Very little is known concerning the structure of such a D , whereas the normal division algebras of smaller index have been completely discussed. In this paper, I will show that *there exists an extension field F^* , obtained by successively adjoining to F the roots of two quadratic equations and one root of a cubic equation, such that the extended algebra D_{F^*} over F^* is cyclic*. This implies that D possesses soluble splitting fields, of degree at most 60. The question whether a given division algebra has soluble splitting fields resembles the question in the commutative case whether a given equation can be solved by means of radicals. Our method is analogous to the Tschirnhaus transformation of an equation. We shall have to solve four equations, of degrees 1, 2, 3, 4, respectively, for 25 parameters. We shall succeed in doing so after adjoining successively algebraic quantities of second and third degree to the ground field. The reason that the corresponding procedure does not work in the case of the solution of equations of the fifth degree is that there we have only five parameters instead of 25 parameters.

Our result for normal division algebras D of index 5 does not answer the question whether any such D itself is cyclic. However, it remains only to investigate algebras D with relatively simple splitting fields, and it may be hoped that this will greatly simplify the work on that problem.

The method given here can also be used for the investigation of normal division algebras of index $m = 3$ and $m = 4$; it yields a proof of the theorems of J. L. M. Wedderburn² and A. A. Albert³ for these cases.

2. Let D be a normal division algebra of index m over a given field F . There exists always a separable splitting field $F(\vartheta)$ of degree m over F . Let $\vartheta_1 = \vartheta, \vartheta_2, \dots, \vartheta_m$ be the conjugates of ϑ with regard to F . We denote the normal field $F(\vartheta_1, \vartheta_2, \dots, \vartheta_m)$ by Ω , and its Galois group by \mathfrak{G} ; and we set $\omega^G = \omega'$, if the number ω of Ω is carried into ω' by the element G of \mathfrak{G} . If $\vartheta_\mu^G = \vartheta_\nu$, we write $\nu = \mu G$. A conjugate triple system $c_{\alpha\beta\gamma}$ in Ω is a system of m^3 numbers $c_{\alpha\beta\gamma}$ ($\alpha, \beta, \gamma = 1, 2, \dots, m$) in Ω , such that the equation

$$c_{\alpha\beta\gamma}^G = c_{\alpha G, \beta G, \gamma G}$$

holds for every G in \mathfrak{G} and for all α, β, γ . Similarly, conjugate double systems $l_{\alpha\beta}$ in Ω are systems of m^2 numbers of Ω for which $l_{\alpha\beta}^G = l_{\alpha G, \beta G}$ holds.

It was shown in a previous paper,⁴ that to D there corresponds a system of m^3 numbers $c_{\alpha\beta\gamma}$ such that

- (a) $c_{\alpha\beta\gamma}$ is a conjugate triple system in Ω ,
- (b) $c_{\alpha\beta\gamma}c_{\alpha\gamma\delta} = c_{\alpha\beta\delta}c_{\beta\gamma\delta}$ ($\alpha, \beta, \gamma, \delta = 1, 2, \dots, m$),
- (c) $c_{\alpha\beta\gamma} \neq 0$ for all α, β, γ .

The system $c_{\alpha\beta\gamma}$ is called the factor system of D . The algebra D is then isomorphic to the algebra D_0 consisting of all matrices of degree m of the form

$$A = (l_{\kappa\lambda}c_{\kappa\lambda 1}) \quad (\kappa, \lambda = 1, 2, \dots, m) \quad (1)$$

where $l_{\kappa\lambda}$ is any conjugate double system in Ω .

3. We form the characteristic polynomial of the matrix A

$$|xI - A| = x^m - h_1x^{m-1} + h_2x^{m-2} - \dots + (-1)^mh_m$$

(I denotes the unit matrix of degree m). A simple computation gives

$$h_r = \sum_{\rho_1 < \rho_2 < \dots < \rho_r}^m, \quad \sum l_{\rho_1\sigma_1} l_{\rho_2\sigma_2} \dots l_{\rho_r\sigma_r} c_{\rho_1\sigma_1 1} c_{\rho_2\sigma_2 1} \dots c_{\rho_r\sigma_r 1} \chi_{\sigma_1\sigma_2\dots\sigma_r} \quad (2)$$

where the inner sum is to be extended over all permutations $\sigma_1, \sigma_2, \dots, \sigma_r$ of $\rho_1, \rho_2, \dots, \rho_r$ and where $\chi(\sigma_1, \sigma_2, \dots, \sigma_r)$ is $+1$ or -1 according as the permutation is even or odd.

We now put in (1)

$$\left. \begin{aligned} l_{\kappa\kappa} &= 0 & (\kappa &= 1, 2, \dots, m), \\ l_{\kappa\lambda} &= (u_0 + u_1\vartheta_\kappa + \dots + u_{m-1}\vartheta_\kappa^{m-1})^{-1} = \varphi(\vartheta_\kappa)^{-1}, & (\kappa \neq \lambda) \end{aligned} \right\} \quad (3)$$

where u_0, u_1, \dots, u_{m-1} are numbers in F which do not all vanish. Obviously, we obtain a conjugate double system. From (2) and (3) it follows that $h_r\varphi(\vartheta_1)\varphi(\vartheta_2)\dots\varphi(\vartheta_m)$ is a homogeneous polynomial P_{m-r} of degree $m-r$ in u_0, u_1, \dots, u_{m-1} :

$$h_r\varphi(\vartheta_1)\varphi(\vartheta_2)\dots\varphi(\vartheta_m) = P_{m-r}(u_0, u_1, \dots, u_{m-1}). \quad (4)$$

We show that the coefficients of P_{m-r} lie in F . We apply an element G of \mathfrak{G} . Then h_r^G equals the corresponding coefficient in the characteristic equation of A^G , the image of A under G . We may, for a moment, consider u_0, u_1, \dots, u_{m-1} as indeterminates which are not changed by G . Put $\kappa G = \kappa', \lambda G = \lambda', 1G = \zeta$. Then we have $A^G = (c_{\kappa'\lambda'\zeta}l_{\kappa'\lambda'})$, and after rearranging the rows and columns using the same permutation both times, we see that A^G is similar to

$$A_\zeta = (c_{\kappa\lambda\zeta}l_{\kappa\lambda}) \quad (\kappa, \lambda = 1, 2, \dots, m).$$

However, A and A_ζ are similar, since $A = QA_\zeta Q^{-1}$ with

$$Q = \begin{pmatrix} c_{1\xi 1} & 0 & \cdot & \cdot & \cdot & 0 \\ 0 & c_{2\xi 1} & \cdot & \cdot & \cdot & 0 \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ 0 & 0 & \cdot & \cdot & \cdot & c_{m\xi 1} \end{pmatrix}$$

as follows at once from the property (b) of the $c_{\alpha\beta\gamma}$. Consequently, A and A^G are similar and have, therefore, the same characteristic equation. This gives $h_r^G = h_r$. Since the factor of h_r in (4) is invariant under G , the same is true for the coefficients of $P_{m-1}(u_0, u_1, \dots, u_{m-1})$. Hence these coefficients lie in F .⁵

The first equation (3) together with (2) shows that $h_1 = 0$. From (4) it follows that

$$P_{m-1}(u_0, u_1, \dots, u_{m-1}) = 0 \quad (5)$$

identically in u_0, u_1, \dots, u_{m-1} . By the special choice of $l_{\kappa\lambda}$ in (3), we disposed of $m^2 - m$ parameters which appear in the general $l_{\kappa\lambda}$. We have, however, the advantage that the equation (5) of degree $m - 1$ is identically satisfied.

4. We now take $m = 5$. We choose u_0, u_1, u_2, u_3, u_4 from the equations

$$P_1(u_0, \dots, u_4) = 0, \quad P_2(u_0, \dots, u_4) = 0, \quad P_3(u_0, \dots, u_4) = 0, \quad (6)$$

which are homogeneous of degrees 1, 2 and 3. After eliminating one indeterminate by means of $P_1 = 0$, we may interpret the remaining u_i as homogeneous coördinates in a 3-dimensional projective space. We have then to find a point on the intersection of a quadric and a cubic surface. We first determine a straight line s on the quadric. This may require the solution of two quadratic equations. After adjoining the roots to F we take the intersection of s with the cubic. Here we may have to solve a cubic equation. The adjunction of a root to F gives a soluble field F^* , of at most degree 12, and in F^* the equations (6) have a non-trivial solution.

We now replace F by F^* . The factor system of D remains the same. The element A of D , corresponding to the choice (3) of the $l_{\kappa\lambda}$ and (6) of u_0, u_1, \dots, u_4 , is different from 0. Its characteristic equation is

$$x^5 - h_5 = 0, \quad (h_5 \text{ in } F^*).$$

Hence $F^*(\sqrt[5]{h_5})$ is a splitting field of D_{F^*} . This implies that D_{F^*} is a cyclic algebra over F^* .⁶

We had to solve two quadratic equations and one cubic equation in order to obtain F^* . If F has not the characteristic 2 or 3, we can obtain a field F^* such that D_{F^*} is cyclic, by successively adjoining three square roots and a cube root to F .

5. The same method can be used in the case of normal division alge-

bras of index $m = 3$. Here we have to consider only the linear equation $P_1 = 0$ in (6). Without any further adjunction, we obtain an element $A \neq 0$ in D , with a characteristic equation $x^3 - h_3 = 0$. This gives Wedderburn's result that all normal division algebras of index 3 are cyclic.

In the case $m = 4$, we again consider only the equation $P_1 = 0$ instead of (6). We obtain an element $A \neq 0$ in D , which has a characteristic equation of the form $x^4 - h_2x^2 - h_4 = 0$. The construction of such an element A forms the main part of Albert's proof of the theorem that every normal division algebra of index $m = 4$ possesses a Galois splitting field of degree 4 and can, therefore, be written as a crossed product, provided the characteristic of F is different from 2.

¹ For references to the theory of algebras, cf. M. Deuring's book: *Algebren (Ergebnisse der Mathematik)*, Berlin, 1935).

² J. L. M. Wedderburn, *Trans. Amer. Math. Soc.*, **22**, 129 (1931).

³ A. A. Albert, *Trans. Amer. Math. Soc.*, **31**, 253 (1929) and *Bull. Amer. Soc.*, **38**, 703 (1932).

⁴ R. Brauer, *Math. Zeitschr.*, **30**, 79 (1929).

⁵ This can also be seen from general considerations concerning simple algebras, without using the explicit form (1) of the elements.

⁶ A. A. Albert, *Trans. Amer. Math. Soc.*, **36**, 885 (1934). For the case of a field F of characteristic 5, cf. A. A. Albert, *Trans. Amer. Soc.*, **39**, 183 (1936).

THE LIFT AND DRAG FUNCTIONS FOR AN ELASTIC FLUID IN TWO DIMENSIONAL IRROTATIONAL FLOW

BY H. BATEMAN

GUGGENHEIM GRADUATE SCHOOL OF AERONAUTICS,
CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated May 2, 1938

1. The lift function Y and the drag function X are defined by the equation

$$\begin{aligned} dX &= (p + \rho u^2)dy - \rho uvdx = pdy + u d\psi = (p + \rho q^2)dy - \rho v d\phi, \\ dY &= \rho uvdy - (p + \rho v^2)dx = v d\psi - p dx = \rho u d\phi - (p + \rho q^2)dx, \end{aligned} \quad (1)$$

where u, v are the component velocities, p is the pressure and ρ is the density at the point x, y . The symbol q^2 is written for $u^2 + v^2$. Putting $x = r \cos \theta$, $y = r \sin \theta$, $v_r = u \cos \theta + v \sin \theta$, $v_\theta = v \cos \theta - u \sin \theta$ we have

$$\begin{aligned} dX &= (p_\theta \sin \theta - \rho v_r v_\theta \cos \theta)dr + (p_r \cos \theta - \rho v_r v_\theta \sin \theta)r d\theta, \\ dY &= (p_r \sin \theta + \rho v_r v_\theta \cos \theta)r d\theta - (p_\theta \cos \theta + \rho v_r v_\theta \sin \theta)dr, \end{aligned} \quad (2)$$

where $p_r = p + \rho v_r^2$, $p_\theta = p + \rho v_\theta^2$.

Generalizing the analysis of Glauert and Lamb we write

$$\begin{aligned}\phi &= V[r \cos \theta + A_0 + A_1 \log r + A_2(\log r)^2 + \dots \\ &\quad + (1/r)\{B_0 + B_1 \log r + B_2(\log r)^2 + \dots\} + \dots] \\ \psi &= \rho_\infty V[r \sin \theta + C_0 + C_1 \log r + C_2(\log r)^2 + \dots \\ &\quad + (1/r)\{D_0 + D_1 \log r + D_2(\log r)^2 + \dots\} + \dots],\end{aligned}\quad (3)$$

where the coefficients A_s, B_s, C_s, D_s are all functions of θ .

Differentiating these expressions with respect to r and θ we find

$$\begin{aligned}v_r &= \frac{\partial \phi}{\partial r} = V[\cos \theta + (1/r)\{A_1 + 2A_2 \log r + 3A_3(\log r)^2 + \dots\} \\ &\quad + (1/r)^2\{(B_1 - B_0) + (2B_2 - B_1) \log r + \dots\} + \dots], \\ v_\theta &= (1/r) \frac{\partial \phi}{\partial \theta} = V[-\sin \theta + (1/r)\{A'_0 + A'_1 \log r + A'_2(\log r)^2 + \dots\} \\ &\quad + (1/r)^2\{B'_0 + B'_1 \log r + B'_2(\log r)^2 + \dots\} + \dots], \\ \rho v_r &= (1/r) \frac{\partial \psi}{\partial \theta} = \rho_\infty V[\cos \theta + (1/r)\{C'_0 + C'_1 \log r + C'_2(\log r)^2 + \dots\} \\ &\quad + (1/r)^2\{D'_0 + D'_1 \log r + D'_2(\log r)^2 + \dots\} + \dots] \quad (4)\end{aligned}$$

$$\begin{aligned}\rho v_\theta &= -\frac{\partial \psi}{\partial r} = \rho_\infty V[-\sin \theta - (1/r)\{C_1 + 2C_2 \log r + 3C_3(\log r)^2 + \dots\} \\ &\quad + (1/r)^2\{D_0 - D_1 + (D_1 - 2D_2)\log r + (D_2 - 3D_3)(\log r)^2 + \dots\} + \dots],\end{aligned}$$

where primes denote differentiations with respect to θ .

We shall endeavor to express as many coefficients as possible in terms of A and C . To find the expressions we shall assume that

$$\begin{aligned}\rho &= \rho_\infty [1 + R_0(1/r) + R_1(1/r) \log r + R_2(1/r)(\log r)^2 + \dots \\ &\quad + S_0(1/r)^2 + S_1(1/r)^2 \log r + S_2(1/r)^2(\log r)^2 + \dots] \quad (5)\end{aligned}$$

where the coefficients R_n, S_n are all functions of θ .

Using this expression for ρ to obtain series for ρv_r and ρv_θ from those for v_r and v_θ and then comparing the chief terms in the two sets of expansions we obtain a set of equations of which only four will be written down here as sufficient for our present needs but it may be mentioned that the other equations may be needed for a later calculation of the moment on a stationary obstacle.

$$\begin{aligned}R_0 \cos \theta + A_1 &= C'_0, & R_1 \cos \theta + 2A_2 &= C'_1, \\ -R_0 \sin \theta + A'_0 &= -C_1, & -R_1 \sin \theta + A'_1 &= -2C_2.\end{aligned}\quad (6)$$

To obtain another set of equations we shall suppose that the pressure and density are connected by the relation $p = f(\rho) - \rho f'(\rho)$ so that if c is the local velocity of sound, $c^2 = dp/d\rho = -\rho f''(\rho)$. We shall suppose,

moreover, that the arbitrary constant in $f'(\rho)$ is chosen so that the equation of Bernoulli is $\frac{1}{2}q^2 = f'(\rho)$. Then by Taylor's expansion, if $a^2 = (dp/d\rho)_\infty = -\rho_\infty f''(\rho_\infty)$, we have

$$\frac{1}{2}q^2 = \frac{1}{2}V^2 + (\rho - \rho_\infty)f''(\rho_\infty) + \dots$$

Substituting the expression for $\rho - \rho_\infty$ and equating coefficients of $(1/r)$, $(1/r) \log r$, etc., we find that

$$V^2(A_1 \cos \theta - A'_0 \sin \theta) = -a^2 R, \quad V^2(2A_2 \cos \theta - A'_1 \sin \theta) = -a^2 R_1 \quad (7)$$

the other equations being omitted though they may be needed in a calculation of the moment.

Writing $A_1 = A(a^2 - V^2 \sin^2 \theta)$, $C_1 = C(a^2 - V^2 \sin^2 \theta)$ we find that

$$A'_0 = -V^2 A \sin \theta \cos \theta - a^2 C, \quad C'_0 = (a^2 - V^2)A - V^2 C \sin \theta \cos \theta. \quad (8)$$

If the total circulation is $-K$, a finite quantity, we find that

$$K = - \int_0^{2\pi} v_\theta r d\theta = -V \int_0^{2\pi} A'_0 d\theta = V^3 \int_0^{2\pi} A \sin \theta \cos \theta d\theta + a^2 V \int_0^{2\pi} C d\theta \quad (9)$$

and that A_1, A_2, \dots must be uniform functions of θ .

Furthermore, if there is no flow of matter to infinity on the whole C_0, C_1, C_2, \dots must be uniform functions of θ and so, in particular,

$$0 = \int_0^{2\pi} C'_0 d\theta \therefore (a^2 - V^2) \int_0^{2\pi} A d\theta = V^2 \int_0^{2\pi} C \sin \theta \cos \theta d\theta. \quad (10)$$

We also find from equations (6) and (7) that

$$\begin{aligned} R_0 &= -V^2(A \cos \theta + C \sin \theta), \\ R_1(a^2 - V^2 \cos^2 \theta) &= V^2 A'_1 \sin \theta - V^2 C'_1 \cos \theta, \\ 2A_2(a^2 - V^2 \cos^2 \theta) &= a^2 C'_1 - V^2 A'_1 \sin \theta \cos \theta, \\ -2C_2(a^2 - V^2 \cos^2 \theta) &= A'_1(a^2 - V^2) + C'_1 V^2 \sin \theta \cos \theta, \end{aligned} \quad (11)$$

$$p = p_\infty + a^2(\rho - \rho_\infty) + \dots$$

$$= p_\infty - \rho_\infty V^2(A_1 \cos \theta - A'_0 \sin \theta)(1/r)$$

$$- \rho_\infty V^2(2A_2 \cos \theta - A'_1 \sin \theta)(1/r) \log r \text{ approximately.}$$

Combining the last equation with equations obtained by forming the squares and products of the series in equations (4) we find that to a first approximation

$$\begin{aligned}
p_r &= p_\infty + \rho_\infty V^2 [\cos^2 \theta + (1/r)(C'_0 \cos \theta + A'_0 \sin \theta) \\
&\quad + (1/r)(\log r)(C'_1 \cos \theta + A'_1 \sin \theta)] \\
\rho v_r v_\theta &= \rho_\infty V^2 [-\sin \theta \cos \theta + (1/r)(A'_0 \cos \theta - C'_0 \sin \theta) \\
&\quad + (1/r)(\log r)(A'_1 \cos \theta - C'_1 \sin \theta)] \quad (12) \\
p_\theta &= p_\infty + \rho_\infty V^2 [\sin^2 \theta + (1/r)(C_1 \sin \theta - A_1 \cos \theta) \\
&\quad + (1/r) \log r (2C_2 \sin \theta - 2A_2 \cos \theta)].
\end{aligned}$$

Hence to a first approximation

$$\begin{aligned}
dX &= dr[(p_\infty + \rho_\infty V^2) \sin \theta + \rho_\infty V^2 C_1(1/r)] \\
&\quad + d\theta [p_\infty r \cos \theta + \rho_\infty V^2(r \cos \theta + C'_0 + C'_1 \log r)] \\
X &= (p_\infty + \rho_\infty V^2)r \sin \theta + \rho_\infty V^2(C_0 + C_1 \log r); \quad (13)
\end{aligned}$$

thus X is a uniform function of θ .

$$\begin{aligned}
-dY &= dr[p_\infty \cos \theta + (1/r) \\
&\quad (C_1 \sin \theta \cos \theta - A_1 \cos^2 \theta + A'_0 \sin \theta \cos \theta - C'_0 \sin^2 \theta) + (1/r) \\
&\quad \log r (2C_2 \sin \theta \cos \theta - 2A_2 \cos^2 \theta + A'_1 \sin \theta \cos \theta - C'_1 \sin^2 \theta) \\
&\quad + d\theta [p_\infty r \sin \theta + \rho_\infty V^2(A'_0 + A'_1 \log r + \dots)] \quad (14) \\
Y &= p_\infty r \cos \theta - \rho_\infty V^2 \{A_0 + A_1 \log r + \dots\}.
\end{aligned}$$

Since A_1 is a uniform function of θ the total lift is $KV\rho_\infty$ as in the theorems of Lord Rayleigh, Kutta, Joukowski and Glauert.

2. We now introduce the functions $\chi = ux + vy - \phi$, $\Omega = \rho uy - \rho vx - \psi$, which occur in the theory of the Legendre transformation of the partial differential equations satisfied by ϕ and ψ . Their approximate expressions are

$$\begin{aligned}
\chi &= r \frac{\partial \phi}{\partial r} - \phi = V[A_1 - A_0 + (2A_2 - A_1) \log r + \dots \\
&\quad + (1/r)\{B_1 - 2B_0 + (2B_2 - 2B_1) \log r + \dots\} + \dots] \quad (15) \\
\Omega &= r \frac{\partial \psi}{\partial r} - \psi = \rho_\infty V[C_1 - C_0 + (2C_2 - C_1) \log r + \dots \\
&\quad + (1/r)\{D_1 - 2D_0 + (2D_2 - 2D_1) \log r + \dots\} + \dots].
\end{aligned}$$

We see that both χ and Ω are generally infinite for large values of r . When r is kept constant Ω is a uniform function of θ but χ is not; the change in χ as θ increases from 0 to 2π is for large values of r equal to K .

It should be remarked that if we write $u = q \cos \omega$, $v = q \sin \omega$, the functions χ and Ω are connected by the equations

$$\frac{\partial \Omega}{\partial \omega} = -q\rho \frac{\partial \chi}{\partial q}, \quad \frac{\partial \chi}{\partial \omega} = q \frac{\partial \Omega}{\partial(\rho q)}. \quad (16)$$

3. If, by means of equations (1) we express dX and dY in terms of $d\phi$ and $d\psi$ we obtain the equations

$$\begin{aligned} p d\phi &= v dX - u dY, \\ (p + \rho q^2) d\psi &= \rho(u dX + v dY), \\ \rho q^2 dX &= p \rho v d\phi + (p + \rho q^2) u d\psi, \\ \rho q^2 dY &= -p \rho u d\phi + (p + \rho q^2) v d\psi \end{aligned} \quad (17)$$

which may be compared with the equations

$$\begin{aligned} d\phi &= u dx + v dy, \\ d\psi &= \rho u dy - \rho v dx, \\ \rho q^2 dx &= \rho u d\phi - v d\psi, \\ \rho q^2 dy &= \rho v d\phi + u d\psi. \end{aligned} \quad (18)$$

Introducing a fictitious elastic fluid in the XY -plane and using large letters for the quantities relating to this fluid except for the density which we denote by σ , we have relations

$$\begin{aligned} P &= -1/p, \quad P + \sigma Q^2 = -1(p + \rho q^2), \quad Q = q/p, \\ C^2 &= \frac{dP}{d\sigma}, \quad 1 - \frac{Q^2}{C^2} = \left(1 - \frac{q^2}{c^2}\right) \left(\frac{p}{p + \rho q^2}\right)^2 \\ \frac{dP}{dQ} &= -\frac{q\rho}{p + \rho q^2} = -Q\sigma. \end{aligned} \quad (19)$$

The last equation, which corresponds to $dp/dq = -q\rho$ shows that P , Q , σ are connected by the same relation as the pressure, velocity and density in an elastic fluid. We also have the relations

$$\begin{aligned} p(p + \rho q^2) dx &= \rho u v dX - (p + \rho u^2) dY, \\ p(p + \rho q^2) dy &= (p + \rho v^2) dX - \rho u v dY, \\ P + \sigma U^2 &= -\frac{p + \rho u^2}{p(p + \rho q^2)}, \quad P + \sigma V^2 = -\frac{p + \rho v^2}{p(p + \rho q^2)}, \\ \sigma UV &= -\frac{\rho uv}{p(p + \rho q^2)} \end{aligned} \quad (20)$$

which show that there is a complete reciprocity between the real fluid and the fictitious fluid. Another correspondence between two elastic fluids may be obtained by introducing two arbitrary constants h , H and writing $x' = x + hY$, $y' = y - hX$, $X' = X + Hy$, $Y' = Y - Hx$. We then have the relations

$$\begin{aligned} v dX' - u dY' &= (p + H) d\phi, \quad \rho u dX' + \rho v dY' = (p + \rho q^2 + H) d\psi \\ v dy' + u dx' &= (1 - hp) d\phi, \quad \rho u dy - \rho v dx' = 1 - h(p + \rho q^2) d\psi. \end{aligned} \quad (21)$$

Writing $\phi' = \phi$, $\psi' = (1 + hH)\psi$ and introducing primed quantities for a fictitious fluid in which ψ' is the stream function and ϕ' the velocity potential, we have the relations

$$\begin{aligned} p' &= \frac{p + H}{1 - ph}, \quad u' = \frac{u}{1 - ph}, \quad v' = \frac{v}{1 - ph}, \quad q' = \frac{q}{1 - ph} \\ \rho' &= \rho \frac{(1 - ph)(1 + Hh)}{1 - h(p + \rho q^2)}, \quad p' + \rho' q'^2 = \frac{p + \rho q^2 + H}{1 - h(p + \rho q^2)} \\ \frac{dp'}{dp} &= \frac{1 + hH}{(1 - ph)^2}, \quad \frac{dp'}{dq'} = -\rho' q' \\ 1 - \frac{q'^2}{c'^2} &= \left(1 - \frac{q^2}{c^2}\right) \frac{(1 - hp)^2}{[1 - h(p + \rho q^2)]^2}. \end{aligned} \quad (22)$$

The last relation shows that $q'^2 > c'^2$ when $q^2 > c^2$. This is to be expected from the general behavior of characteristics in a point transformation.

H. Glauert, *Proc. Roy Soc. London*, **118**, 133, (1928).

H. Lamb, *Aeronautical Research Committee, R. M.*, 1156 (Ae 321) (1928).

E. Postolesi, *Le Alte Velocità in Aviazione*, 283, Rome (1936).

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

July 15, 1938

Number 7

Copyright 1938 by The National Academy of Sciences

THE DISTRIBUTION OF GENE FREQUENCIES UNDER IRREVERSIBLE MUTATION

BY SEWALL WRIGHT

DEPARTMENT OF ZOÖLOGY, THE UNIVERSITY OF CHICAGO

Communicated June 13, 1938

Under reversible mutation, the frequency (q) of a gene, subject to systematic evolutionary pressure (Δq) and to the accidents of sampling in a limited population (N diploid individuals), varies according to a certain distribution ($\varphi(q)$) discussed in previous papers.^{1,2,3} If mutation is irreversible, the distribution curve for such genes should attain constancy of form, but all class frequencies should fall off at a uniform rate (K) as genes drift irreversibly into fixation. The purpose of the present paper is to broaden somewhat the treatment in this latter case.

As previously shown² the rate of fixation is approximately half the frequency, $f(1 - 1/2N)$, in the subterminal class. Thus with

$$\int_0^1 \varphi(q) dq = 1$$

$$K = 1/2 f(1 - 1/2N) = \frac{\varphi(1 - 1/2N)}{4N} \text{ approximately.} \quad (1)$$

The changes in the mean ($\bar{q} = \int_0^1 q\varphi(q) dq$) and the variance ($\sigma_q^2 = \int_0^1 (q - \bar{q})^2 \varphi(q) dq$) of gene frequencies, due to fixation in one generation, may be expressed as follows in terms of the systematic evolutionary pressure, Δq , and the variation due to sampling, $\sigma_{\Delta q}^2$

$$\int_0^1 \Delta q \varphi(q) dq = K(1 - \bar{q}). \quad (2)$$

$$\int_0^1 (q + \Delta q - \bar{q})^2 \varphi(q) dq + \int_0^1 \sigma_{\Delta q}^2 \varphi(q) dq = (1 - K)\sigma_q^2 + K(1 - \bar{q})^2. \quad (3)$$

The latter can be reduced to following, ignoring a negligible term in $(\Delta q)^2$

$$2 \int_0^1 (q - \bar{q}) \Delta q \varphi(q) dq + \int_0^1 \sigma_{\Delta q}^2 \varphi(q) dq = K[(1 - \bar{q})^2 - \sigma_q^2]. \quad (4)$$

If the conditions are such that under equilibrium, with reverse mutation at an indefinitely low rate, there is no important accumulation of genes in the class $q = 1$, complete irreversibility of mutation should make no appreciable difference in the form of distribution. The demonstration of the formula for this case ($K = 0$) can be put in a very simple form.

$$\text{Let } \int \Delta q \varphi(q) dq = \chi(q). \quad (5)$$

Equations (2) and (4) can be written as follows, putting $K = 0$,

$$\chi(1) - \chi(0) = 0. \quad (6)$$

$$\int_0^1 \chi(q) dq - [\bar{q}\chi(0) + (1 - \bar{q})\chi(1)] - \frac{1}{2} \int_0^1 \sigma_{\Delta q}^2 \varphi(q) dq = 0. \quad (7)$$

Equation (7) is obviously satisfied by the following

$$\chi(q) - [\bar{q}\chi(0) + (1 - \bar{q})\chi(1)] - \frac{1}{2} \sigma_{\Delta q}^2 \varphi(q) = 0. \quad (8)$$

This means little, until it is shown that (8) also satisfies (6).

As there can be no sampling variance in homallelic populations, $\sigma_{\Delta q}^2 = 0$ if $q = 0$ or $q = 1$. Equation (8) reduces to (6) if $q = 0$ or $q = 1$ and both $\varphi(0)$ and $\varphi(1)$ are finite. Equation (8), therefore, satisfies the condition of constancy of the mean as well as that of constancy of the variance.

$$\varphi(q) = \frac{2[\chi(q) - \chi(1)]}{\sigma_{\Delta q}^2}. \quad (9)$$

$[\chi(q) - \chi(1)]$ can be evaluated as follows, using (5) and (9),

$$d \log [\chi(q) - \chi(1)] = \frac{d\chi(q)}{\chi(q) - \chi(1)} = \frac{2 \Delta q \varphi(q) dq}{\sigma_{\Delta q}^2 \varphi(q)}, \quad (10)$$

$$\chi(q) - \chi(1) = \frac{C}{2} e^{2 \int \frac{\Delta q dq}{\sigma_{\Delta q}^2}}, \quad (11)$$

$$\varphi(q) = \frac{C e^{2 \int (\Delta q / \sigma_{\Delta q}^2) dq}}{\sigma_{\Delta q}^2}. \quad (12)$$

This is the desired expression for the distribution in terms of Δq and $\sigma_{\Delta q}^2$.

Putting $\sigma_{\Delta q}^2 = \frac{q(1-q)}{2N}$, its value in a population of N diploid indi-

viduals, it is the same as the formula given previously³ (except for the value of the coefficient C).

If K is not zero, this method does not appear to lead to a usable general expression which satisfies (1), (2) and (4). An expression for the rate of decay can, however, be obtained from (2). Let v be the rate of mutation, and let \bar{W} be the mean selective value of genotypes. As shown previously³

$$\Delta q = v(1 - q) + q(1 - q) \frac{d \log \bar{W}}{2dq}. \quad (13)$$

$$\text{From (2)} \quad K = v + \frac{1}{2(1 - \bar{q})} \int_0^1 q(1 - q) \varphi(q) d \log \bar{W}. \quad (14)$$

If there is no selection ($\bar{W} = 1$)

$$K = v. \quad (15)$$

The distribution (16) for this case was obtained in a previous paper² by substitution of $\Delta q = v(1 - q)$ in what is equation (18) of the present paper. It can easily be verified that (16) also satisfies (1), (2) and (4).

$$\varphi(q) = 4Nvq^{4Nv-1}. \quad (16)$$

The most important cases are probably those in which the size of population is so small that recurrent mutation has no effect on the form of distribution (v much less than $1/4N$). The selection pressure for any degree of dominance can be written sufficiently accurately

$$\Delta q = (s + tq)q(1 - q). \quad (17)$$

The condition that the frequency of any class of gene frequencies, q_c , be reconstructed after each generation, except for a uniform decay at rate K can be represented as follows,² using $p = 1 - q$ for brevity.

$$(1 - K)\varphi(q_c) = A \int_0^1 (q + \Delta q)^{2Nq_c} (p - \Delta q)^{2Np_c} \varphi(q) dq$$

$$\text{where} \quad A = \frac{\Gamma(2N)}{p_c q_c \Gamma(2Np_c) \Gamma(2Nq_c)}. \quad (18)$$

If v is so small that practically all genes are fixed in the class $f(0)$, K may be ignored in determining the form of the distribution for unfixed genes. Substituting the value of Δq from (17)

$$\varphi(q_c) = A \int_0^1 q^{2Nq_c} p^{2Np_c} [1 + p(s + tq)]^{2Nq_c} [1 - q(s + tq)]^{2Np_c} \varphi(q) dq. \quad (19)$$

The following approximations may be used

$$[1 + p(s + tq)]^{2Nq_c} = e^{2Nq_c p(s + tq)} [1 - Nq_c p^2 (s + tq)^2]. \quad (20)$$

$$[1 - q(s + tq)]^{2Np_c} = e^{-2Np_cq(s+tq)} [1 - Np_cq^2(s + tq)^2]. \quad (21)$$

The product of expressions (20) and (21) is approximately

$$e^{2Ns(q_c - q) + Nt(q_c^2 - q^2) - Nt(q_c - q)^2} \{1 - N(s + tq)^2[p_cq_c + (q_c - q)^2]\}. \quad (22)$$

Since the random deviations of q have the variance, $\sigma_{\Delta q}^2 = \frac{pq}{2N}$, $(q_c - q)^2$

is of the order $1/2N$. The term $N(s + tq)^2(q_c - q)^2$ is thus negligibly small compared with $N(s + tq)^2p_cq_c$ which itself is as small a term as it is necessary to consider. The former may thus be ignored. The constant and variable gene frequencies are separable in the exponential term in (22) except in the term $e^{-Nt(q_c - q)^2}$. The exponent in this case is smaller than $-t$ and the term can be written $[1 - Nt(q_c - q)^2]$ with sufficient accuracy. Equation (19) can now be written as follows:

$$\varphi(q_c) = Ae^{2Ns q_c + Nt q_c^2} \int_0^1 q^{2Nq_c} p^{2Np_c} e^{-2Ns q - Nt q^2} [1 - N(s + tq)^2 p_c q_c - Nt(q_c - q)^2] \varphi(q) dq. \quad (23)$$

Let
$$\varphi(q) = \frac{e^{2Ns q + Nt q^2}}{q(1-q)} (C_0 + C_1 q + C_2 q^2 \dots). \quad (24)$$

This entirely eliminates the exponential terms, leaving (23) in a form which can be solved, using the following sufficiently accurate formula in which terms of the order $\frac{1}{N^2}$ are ignored.

$$\frac{\Gamma(2N)}{\Gamma(2Np_c)\Gamma(2Nq_c)} \int_0^1 q^{2Nq_c-1+x} p^{2Np_c-1} dq = q_c^x \left[1 - \frac{x(x-1)}{4N} \right] + q_c^{x-1} \left[\frac{x(x-1)}{4N} \right]. \quad (25)$$

The resulting coefficients of the powers of q_c on the right side may be equated to those on the left side leading to the following general expression (in which $C_{-1} = C_{-2} = C_{-3} = 0$).

$$C_m = \frac{m(m+1)}{4N} C_{m+1} + C_m - \frac{C_{m-1}}{2} (2Ns^2 + t) - C_{m-2} (2Nst) - C_{m-3} Nt^2. \quad (26)$$

The higher coefficients can all be expressed in terms of C_0 and C_1 and substituted in (24). By letting $C_1 = 2NsC_0 + D$ the terms for which C_0 is the coefficient can be condensed into exponential form. It will be convenient to substitute C for C_0 .

$$\varphi(q) = \frac{e^{2Nsq + Ntq^2}}{q(1-q)} \left[Ce^{2Nsq + Ntq^2} + Dq\psi(q) \right], \quad (27)$$

where

$$\begin{aligned} \psi(q) = & 1 + \frac{(2Nsq)^2}{|3|} + \frac{(2Nsq)^4}{|5|} + \frac{(2Nsq)^6}{|7|} \dots \quad (28) \\ & + (2Ntq^2) \left(\frac{1}{|3|} + \frac{(2Nsq)}{|3|} + \frac{2(2Nsq)^2}{|5|} + \frac{2(2Nsq)^3}{|5|} + \frac{3(2Nsq)^4}{|7|} \dots \right) \\ & + (2Ntq^2)^2 \left(\frac{7}{|5|} + \frac{2(2Nsq)}{|5|} + \frac{69(2Nsq)^2}{|7|} \dots \right) \\ & + (2Ntq^2)^3 \left(\frac{27}{|7|} \dots \right) + \dots \end{aligned}$$

If $D = 0$

$$\varphi(q) = \frac{Ce^{4Nsq + 2Ntq^2}}{q(1-q)}. \quad (29)$$

This is the case of equilibrium under reversible mutation or irreversible mutation opposed by sufficiently strong selection as can be seen by substituting $\Delta q = (s + tq)q(1 - q)$, $\sigma_{\Delta q}^2 = \frac{q(1-q)}{2N}$ in (12). They agree except in the coefficient.

The case of irreversible mutation with fixation occurring at a low rate, can be found from (1) and (2), assuming that nearly all genes are in one of the homallelic classes. It should be noted that the formula for $\varphi(q)$ only applies where K is of lower order than $1/2N$, $2Ns^2 + t$ in (26). Mutations to the class $q = 1/2N$ contribute the amount $2Nvf(0) = \frac{1}{2}f(1/2N)$ and these contribute to the change of mean by the amount $f(1/2N)/4N$. The mean, however, must be so low that the term $K(1 - \bar{q})$ may be written \bar{K} sufficiently accurately. The following relations are all approximate:

$$\int_0^1 \Delta q \varphi(q) dq + \frac{f(1/2N)}{4N} = K = \frac{f(1 - 1/2N)}{2}. \quad (30)$$

$$f(1/2N) = C \left[1 + \frac{1}{2N} + 2s \right] + \frac{D}{2N}. \quad (31)$$

$$f(1 - 1/2N) =$$

$$C[e^{4Ns + 2Nt} (1 + 1/2N - 2s - 2t)] + D[e^{2Ns + Nt} (1 - s - t)\psi(1 - 1/2N)]. \quad (32)$$

$$\int_0^1 \Delta q \varphi(q) dq = C \left[\frac{e^{4Ns + 2Nt}}{4N} - \frac{1}{4N} \right] + \frac{Ds}{2} + \frac{Dt}{3}. \quad (33)$$

Substituting (31), (32), (33) in (30) and substituting $\psi(1)$ for $\psi(1 - 1/2N)$ (leading terms in difference, $t/3$, $\frac{2Ns^2}{3}$)

$$D = - \frac{Ce^{2Ns + Nt}}{\psi(1)}. \quad (34)$$

With practically all genes in the class $q = 0$, $f(0) = 1 = \frac{f(1/2N)}{4Nv} = \frac{C}{4Nv}$.

Thus $C = 4Nv$ approximately.

$$\varphi(q) = \frac{4Nve^{4Nsq + 2Ntq^2}}{q(1 - q)} \left[1 - \frac{e^{2Ns(1-q) + Nt(1-q^2)} q \psi(q)}{\psi(1)} \right]. \quad (35)$$

For sufficiently small values of Ns and Nt we may take $\psi(q) = 1 + 1/3Ntq^2$ and represent the exponentials by the first two terms of their expansions. The following shows how the hyperbolic distribution $4Nv/q$ is modified by weak selection (s positive for favorable mutation, negative for unfavorable mutation).

$$\varphi(q) = \frac{4Nv}{q} [1 + 2Nsq + 2/3Ntq(2q - 1)]. \quad (36)$$

The rate of fixation (K) of genes can be found from the left member of (30) (in which inaccuracies in the evaluation of D have less effect than in the right member).

$$K = v \left[e^{4Ns + 2Nt} - \frac{(2Ns + 4/3Nt)e^{2Ns + Nt}}{\psi(1)} \right]. \quad (37)$$

This reduces to $v(1 + 2Ns + 2/3Nt)$ for such small values of Ns and Nt as implied in (36). It is to be noted that irreversible mutation should ultimately lead to fixation of the mutant even when opposed by selection (s negative) but the rate is exceedingly slow unless Ns and Nt are small.

In the special case of genic selection ($t = 0$)

$$\psi(q) = \frac{e^{2Nsq} - e^{-2Nsq}}{4Nsq}. \quad (38)$$

$$\varphi(q) = \frac{4Nv}{(1 - e^{-4Ns})} \frac{[1 - e^{-4Ns(1-q)}]}{q(1 - q)}. \quad (39)$$

An essentially similar derivation of this formula has been given previously by the author² and a different one by Fisher.⁴ In this case $K = \frac{4Nvs}{1 - e^{-4Ns}}$ approaching $v(1 + 2Ns)$ as $4Ns$ decreases.

The question of the chance of fixation of an individual mutation must be distinguished from the rate of fixation (K) under recurrent mutation. The chance of fixation is given by the ratio $f(1 - 1/2N)/f(1/2N) = K/2Nv$. In the case of no dominance, this gives $2s/(1 - e^{-4Ns})$ or approximately $2s$ for favorable mutations occurring in a large population, in agreement with Fisher.⁴ For indifferent factors it is $1/2N$. Unfavorable mutations have a chance of fixation $2s/(e^{4Ns} - 1)$ but this is small unless $4Ns$ is small.

The results presented here bear on the possibility of a course of evolutionary change determined by mutation pressure, a process which at first sight seems the most obvious implication of modern genetics. The possibility does indeed exist but requires either an almost complete indifference of the mutation with respect to adaptive value or else a very small effective size of population over a long period of time. The most important case in which mutation pressure seems likely to be a major factor is that of extreme degeneration or elimination of organs that have ceased to be useful.^{5,6} The degeneration of the eyes and loss of pigment of cave forms is an example of a case in which the conditions make it especially probable that mutation pressure is a real factor. In all of these cases, however, the likelihood that various direct and indirect effects of selection may also play a rôle should not be ignored.⁵

It should be noted that while the average rate of fixation of irreversible mutations is low, the large element of chance with respect to *which* mutations become fixed in each particular case makes this a greater factor in the diversification of small isolated populations than is at first apparent. Indeed there may be much diversification of gene frequencies among such populations under conditions in which there is no appreciable systematic tendency toward fixation of the sort investigated here.

¹ Wright, S., *Amer. Nat.*, **63**, 556-561 (1929).

² Wright, S., *Genetics*, **16**, 97-159 (1931).

³ Wright, S., *Proc. Nat. Acad. Sci.*, **23**, 307-320 (1937).

⁴ Fisher, R. A., *Proc. Roy. Soc. Edinburgh*, **50**, 205-220 (1930).

⁵ Wright, S., *Amer. Nat.*, **63**, 274-279 (1929).

⁶ Haldane, J. B. S., *Ibid.*, **67**, 5-19 (1933).

AUXIN IN ISOLATED ROOTS GROWING IN VITRO¹³

By J. VAN OVERBEEK AND JAMES BONNER

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA
INSTITUTE OF TECHNOLOGY

Communicated June 15, 1937

Up to the year 1933 opinion was divided as to whether or not auxin is present in the root. In this year Boysen-Jensen¹ conclusively demonstrated that auxin is present in the normal root. With respect to the isolated root, however, Fiedler² has reported that auxin, although initially present, disappears completely and in general within 24 hours, when the root is cultivated *in vitro*. Nagao³ on the other hand, has shown that auxin may be recovered from isolated roots after 6 days' cultivation *in vitro*. The object of the present work has been primarily to establish, with the aid of improved techniques, whether or not auxin is actually present in isolated roots cultivated *in vitro*. A second but related problem of whether or not auxin is *produced* by such roots will be dealt with in a later communication although experiments of a preliminary nature indicate that it is not produced but is merely carried along in the tip of the isolated root.

Methods.—Pea roots were cultivated *in vitro* under the conditions which have been described elsewhere.⁴ Tips ca. 4 mm. long were removed from the roots of germinating pea seeds⁵ and cultured under strictly sterile conditions in 10 cm. Petri dishes in a basic nutrient solution containing sucrose, inorganic salts, vitamin B₁ (0.1 mg. per liter) and varying amounts of certain amino acids. After the roots had grown for one week and were ca. 70 mm. long, they were subcultured by removal of a 10 mm. tip to fresh medium. If the roots were to be further subcultured this procedure was repeated at weekly intervals. Since lateral roots are formed only after approximately 10 days' culture *in vitro* under these conditions, these experiments are uncomplicated by the presence of more than one growing point per root.

The technique of the auxin extraction and determination has been described in detail elsewhere.⁶ The material to be extracted was placed in highly purified ether, in the cold room (ca. 2°C.) for 15 to 30 hours. The material was neither ground nor acidified⁷ since either of these procedures may result in a partial destruction of auxin. The ether extract was then evaporated to dryness and taken up in a known amount of hot agar (1.5%). This agar was in turn analyzed for auxin by means of the *Avena* test. For each determination 24 test plants were used. In order that the auxin content of the roots might be expressed in "indole-acetic acid equivalents," comparable controls with this substance were also run. In general

two concentrations, one of 20 gamma and one of 7 gamma per liter were used for these controls. Each control was also run with 24 test plants. A blank with plain agar blocks was also included in each test. In an earlier paper⁸ it has been pointed out that the *Avena* curvature-concentration curve often does not pass through the origin, but that it intersects the abscissa. This means that the curvature is not directly proportional to the concentration. There is a certain "threshold concentration" before a curvature is obtained. This threshold concentration, which is in general about 5 gamma per liter, was determined for most of the tests and was applied to the subsequent calculations. It was, however, found that if this threshold concentration is taken into account, the relationship between auxin concentration and *Avena* curvature can be expressed by a straight line,⁹ at least under the conditions of these experiments.

The auxin content of the roots was calculated according to the following formula:

$$\text{auxin concentration} = \frac{(C \times I_1^\circ + O) \times V_{\text{agar}}}{W} \quad \begin{array}{l} \text{gamma indole acetic acid} \\ \text{equivalents per kg. fresh} \\ \text{weight} \end{array}$$

C = the average curvature obtained in the *Avena* test (extracted material)

I_1° = indole acetic acid concentration giving an increase of 1° in the *Avena* test, in gamma per liter

O = threshold concentration, gamma indole acetic acid per liter

V_{agar} = volume of agar in which the dry extract was taken up, cc.

W = fresh weight of material under investigation, gms.

Auxin Concentration in Roots.—Table 1 shows that under the conditions of our experiments auxin is present in isolated pea roots cultivated *in vitro* even after three weeks, and consequently, after 2 subcultures. There can then be no doubt but that auxin may be present in relatively large amounts, even in the cultured root. Fiedler,³ as mentioned above, failed to find auxin in isolated roots (*Zea*, *Pisum*, *Vicia*) after more than 48 hours' growth *in vitro*. That Fiedler failed to extract auxin from *Zea* roots of any age, is undoubtedly due to the extraction technique which he employed. It has been shown elsewhere⁶ that auxin cannot in general be obtained from *Zea* if acid is employed in the extraction (acid was used by Fiedler). It is shown in table 2 that with the present extraction technique, auxin can be obtained from the roots of sterile, germinating *Zea* seeds. The amount present in these roots is smaller than that present in *Pisum* roots of the same length (table 2). It has been shown earlier⁶ that the shoots of *Zea* seedlings, also, contain less auxin than do the shoots of corresponding *Pisum* seedlings. The failure of Fiedler to extract auxin

from isolated pea roots cannot, however, be satisfactorily explained, although this also may have been due to the different techniques employed.

Nature of the Hormone Present.—It was of importance to ascertain whether the auxin of these roots was auxin-a, auxin-b or hetero-auxin (indole acetic acid). This may conveniently be determined by the differential acid-alkali destruction test of Kögl, Haagen-Smit and Erxleben.¹⁰

TABLE 1

AUXIN CONCENTRATIONS FOUND IN ISOLATED ROOTS GROWING *in vitro*, GAMMA "INDOLE ACETIC ACID EQUIVALENTS" PER KG. FRESH WEIGHT. THE CULTURES WERE ANALYZED ONE WEEK AFTER THEY WERE STARTED. CULTURES OF "ALASKA" ROOTS ARE INDICATED BY A, THOSE OF "PERFECTION" BY P. I, II, III, ETC., ADDED TO A OR P INDICATE RESPECTIVELY THE FIRST CULTURE, SECOND CULTURE (FIRST SUBCULTURE), THIRD CULTURE, ETC. THUS THE P III CULTURE AT THE TIME IT WAS ANALYZED HAD BEEN CONTINUED THREE WEEKS SINCE THE INITIAL ROOT TIP WAS CUT FROM THE EMBRYO

EXPT. NUMBER	CULTURE	γ /KG.	REMARKS
80315	A I	1.69	
80413	A I	2.24	no tip
80418	A II	5.88	
...	...	7.48	
80429	A II	6.40	
80517	roots 4 mm. long from embryo, P 0	22.3	
80315	P I	2.26	
80315	P II	2.28	
80331	P II	2.45	
80418	P III	4.12	

TABLE 2

AUXIN CONCENTRATION AND CONTENT OF 1-CM.-LONG ROOTS OF GERMINATING *Zea*, AND *Pisum* SEEDS. GAMMA "INDOLE ACETIC ACID EQUIVALENTS"

EXPT. NUMBER	PLANTS	C	$(C \times I_1^0 + 0)$	V_{AUXIN}	W	γ /KG.	γ /PER ROOT
80603	<i>Zea</i>	3.0	9.4	0.5	2.60	1.64	20.6×10^{-6}
80523	Alaska	10.6	23.3	10.0	6.60	36.0	300.0×10^{-6}
80523	Perfection	7.8	18.0	7.5	4.00	35.3	268.0×10^{-6}
		3.5	9.8	15.0	4.00		

This test is based on the facts (a) that indole acetic acid is heat stable to alkali but is destroyed by acid, (b) that auxin-a is stable to acid but is destroyed by alkali and (c) that auxin-b is unstable to both acid and alkali. Three separate tests of this nature showed that the auxin extracted from pea roots was completely destroyed by alkali, unaffected by acid and must hence have been auxin-a. It might be mentioned in this connection that Heyn¹¹ has shown by another method that the auxin of the roots of germinating *Vicia* seeds is also auxin-a. It might also be noted, however, that one experiment made with *non-sterile* roots of pea seedlings showed the presence of large amounts of indole acetic acid, presumably formed through

the action of microorganisms. Reserve must therefore be exercised in the interpretation of experiments dealing with the auxin relations of non-sterile roots.

The experimental procedure was as follows: A large amount (45 gms.) of roots which had been one week in culture and most of which were without tips (tips had been removed for the subsequent culture) was extracted with ether in the usual fashion, and the extract divided into 4 equal parts. One part was analyzed immediately for the determination of the amount of auxin originally present. The other three portions were placed in flasks and the ether removed completely by distillation. To one sample was then added 3 cc. of 5% HCl; to the second sample, 3 cc. of 0.5 normal NaOH and to the third sample 3 cc. of distilled water. The samples were then refluxed on a boiling water bath for 15 minutes, cooled, neutralized (slightly acid) and extracted with ether. This ether was then tested in the manner described above. The results of one experiment are summarized

TABLE 3
DETERMINATION OF THE NATURE OF AUXIN IN STERILE, ISOLATED ROOTS GROWING
in Vitro. EXPT. NUMBER 80509

REFLUXED WITH	C	$(C \times 1.1^{\circ} + 0)$	V_{agar}	W	$\gamma/\text{mg.}$
HCl	2.6	7.7	0.6	$\frac{1}{4} \times 44.6$	0.420
NaOH	+0.9	0	0.6	$\frac{1}{4} \times 44.6$	0
H ₂ O	2.0	6.9	0.6	$\frac{1}{4} \times 44.6$	0.378
not refluxed	2.3	7.5	1.0	$\frac{1}{4} \times 44.6$	0.681

in table 3. It is of interest to note that the entire analysis was carried out with 30 millionths of a milligram "indole acetic acid equivalents." This corresponds, according to Kögl, Haagen-Smit and Erxleben,¹² to 15 millionths of a mg. of auxin-a.

Distribution of Auxin in the Root.—Table 4 shows that pea roots both at the end of the first culture, i.e., one week *in vitro*, and at the end of the second culture, i.e., two weeks *in vitro*, exhibit a very pronounced gradient of auxin concentration from the tip toward the base. This gradient is much steeper than that found by Thimann⁷ for roots of *Avena* seedlings, but is similar to that found by Boysen-Jensen¹ for roots of germinating *Zea* and *Vicia* seeds.

Is Auxin Produced by Isolated Pea Roots in Vitro?—This question will be treated in detail in a later communication. However, the present experiments permit of a comparison of the total amount of auxin per average initial root tip (the 4 mm. tip cut from the germinating seed) with the total amount of auxin per average root after it has been two weeks in culture (and has hence been subcultured once). The average initial root tip (4 mm.) contains 137×10^{-6} gamma indole acetic acid equivalents. The total auxin content of the average root at the end of the second week is

TABLE 4

DISTRIBUTION OF AUXIN-A IN STERILE, ISOLATED ROOTS GROWN *in Vitro*, GAMMA "INDOLE ACETIC ACID EQUIVALENTS" PER KG. FRESH WEIGHT

EXPT. NUMBER	CULTURE	PART	LENGTH (AVERAGE)	C	(C × I ₁ ⁰ + 0)	V _{agar}	W	γ/KG.
80524	P I	tip	15 mm.	11.2	25.3	0.5	1.001	12.7
		middle	15 mm.	2.1	8.9	0.5	2.003	2.22
		base	24 mm.	15.9	34.0	0.5	7.609	2.23
80511	P II	tip	15 mm.	2.6	8.7	0.6	0.856	6.10
		middle	25 mm.	1.9	7.5	0.4	2.500	1.20
		base	25 mm.	1.1	6.0	0.5	8.900	0.337
80516	P II	tip	15 mm.	5.3	18.8	0.5	1.020	9.2
		middle	15 mm.	4.6	16.4	0.5	2.695	3.04
		base	20 mm.	3.2	12.0	0.5	8.490	0.71

70×10^{-6} gamma indole acetic acid equivalents. Thus although the root at the end of the second week does *contain* auxin, it appears to contain substantially less of this substance than does the initial tip.

Summary.—(1) Isolated *Pisum* roots cultivated *in vitro* were found to contain auxin for at least three weeks after the original tip was removed from the germinating seeds. (2) This auxin obtained from roots under sterile conditions has been shown to be auxin-a. (3) A steep auxin gradient was found in these isolated roots, the highest concentration being found in the tip. (4) Roots after two weeks' cultivation *in vitro* appear to contain less auxin than did the initial root tips.

¹ Boysen-Jensen, P., *Planta*, 19, 354 (1933).

² Fiedler, H., *Zeit. Bot.*, 30, 385 (1936).

³ Nagao, M., *Sci. Rep. Tohoku Imp. Univ.*, 12, 191 (1937).

⁴ Bonner, J., and Addicott, F., *Bol. Gaz.*, 99, 144 (1937).

⁵ Pea seeds of the variety "Perfection," supplied by the Ferry-Morse Seed Co., San Francisco, were used. Seeds of the variety "Alaska" were used in a few experiments with substantially the same results.

⁶ van Overbeek, J., *Proc. Nat. Acad. Sci.*, 24, 42 (1938) and *Bol. Gaz.* (1938) (in press).

⁷ Thimann, K. V., *Jour. Gen. Physiol.*, 18, 23 (1934).

⁸ van Overbeek, J., *Ibid.*, 20, 283 (1936).

⁹ Compare: Thimann, K. V., and Schneider, C. L., *Amer. Jour. Bot.*, 25, 270 (1938).

¹⁰ Kögl, F., Haagen-Smit, A., and Erxleben, H., *Zeit. Physiol. Chem.*, 228, 104 (1934).

¹¹ Heyn, A. N. J., *Proc. Kon. Akad. Wetenschap., Amsterdam*, 38, 1074 (1935).

¹² Kögl, F., Haagen-Smit, A., and Erxleben, H., *Zeit. Physiol. Chem.*, 228, 90 (1934).

¹³ Report of work carried out with the aid of the Works Progress Administration, Official Project Number, 465-03-3-342, Work Project N-9199.

*THE MECHANISM OF HEARING AS REVEALED THROUGH
EXPERIMENT ON THE MASKING EFFECT OF THERMAL
NOISE*

BY HARVEY FLETCHER

BELL TELEPHONE LABORATORIES

Read before the Academy April 26, 1938

Considerable information concerning the dynamical properties of the hearing mechanism can be obtained from physical measurements on audition. In fact, probably more precise information can be obtained by such measurements than from data obtained by animal experimentation, or from data on post mortem sections of human temporal bones.

It is well known that when a person is immersed in a noise his ability to hear other sounds is decreased. The noise is said to produce a masking effect. Before describing the experiments on the masking effect of thermal noise I will first describe the method of obtaining thermal noise and how it is quantitatively defined.

In an electrical conductor there is a statistical variation of the electrical potential difference between its two ends which is due to the thermal agitation of the atoms, including the electrons. This fluctuating voltage may be amplified by means of a vacuum tube amplifier. Such an electrical disturbance is called an electrical thermal noise. If this electrical thermal noise is sent through a telephone receiver, or a loud speaker, it is then converted into acoustical thermal noise. Such an acoustical thermal noise was used in the masking experiments discussed in this paper.

Let I_f be defined as the intensity per cycle of thermal noise using 10^{-16} watts* per square centimeter as a unit of intensity. If ΔI is the intensity due to the thermal noise in the band of frequencies between f and $f + \Delta f$, then

$$I_f = \frac{\Delta I}{\Delta f} \quad (1)$$

The spectrum level B in decibels is defined by

$$B = 10 \log I_f \quad (2)$$

In general I_f and B will vary with the frequency. The curve showing the variation of B is called a spectrogram of the noise.

Let us now examine more closely the relationship between the intensity of the noise and its masking effects. When a thermal noise having a given spectrogram is impressed upon the listener's ears it causes the hearing mechanism to vibrate some parts more and other parts less. These vibrations stimulate the nerve endings. The ear has a selective action for

sounds of different frequencies so that the amount of agitation or stimulation given any set of nerve endings will depend upon their position.

It is well known that the nerve endings responsible for auditory effects are located along the basilar membrane. According to the measurements of anatomists this membrane varies in width from 0.2 millimeter at the oval window end to 0.5 millimeter at the helicotrema end, and is about 30 millimeters long. It will be seen that it is about 100 times as long as it is wide. Therefore, a single coördinate x will define with sufficient accuracy the position of a patch of nerve endings. Then, if we take one per cent of the length it will contain a patch of nerve endings about as long as it is wide.

To make this concept more concrete, a spiral line having approximately the same shape as the human cochlea along which the basilar membrane runs is shown in figure 1. The numbers along the spiral line give the ap-

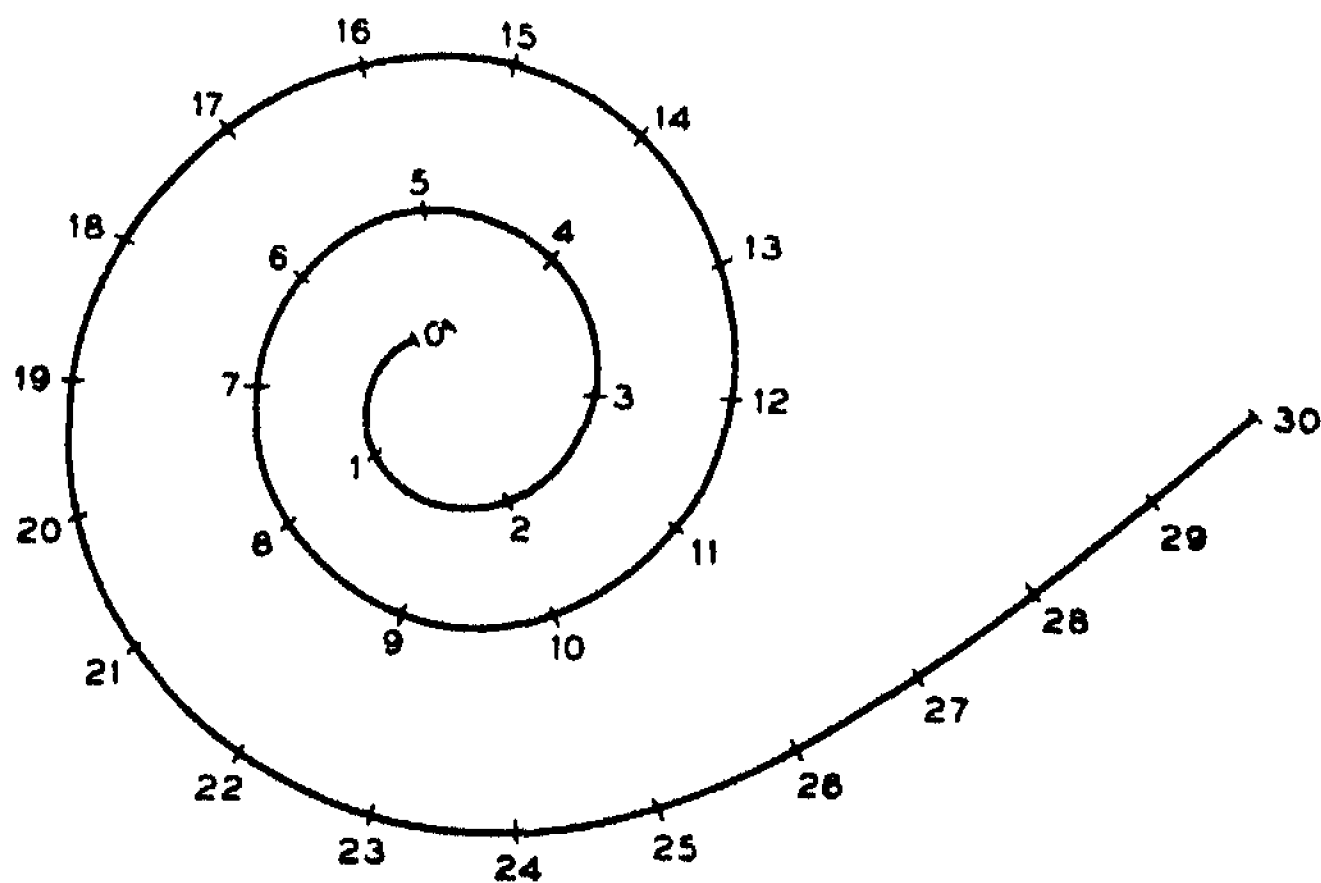


FIGURE 1

proximate distances in millimeters as you go from the helicotrema to the oval window. These dimensions vary in the ears of different persons. Also, the density of the nerve endings, that is, the number of endings per millimeter, is not accurately known. To avoid the necessity of using uncertain anatomical data, the position coördinate x will not be taken in millimeters from the helicotrema but as the per cent of total nerve endings that is passed over in going from the position of maximum stimulation for the lowest audible frequencies (the helicotrema) to a position designated by the coördinate x . The position coördinate thus defined is equal to zero at the helicotrema end and equal to 100 at the oval window end. For example, as will be shown later, the position x of maximum stimulation for a pure tone of frequency 5000 cycles per second is equal approximately to 75. This means that 75 per cent of the nerve endings are in the direction toward the helicotrema and 25 per cent in the direction toward the oval window.

Let us designate the distance in millimeters from the helicotrema as x' . There is then a simple relation between the position coördinate x and the distance in millimeters x' if we know the density σ , that is, the number of nerve endings per millimeter at each distance x' . This relation is given by

$$x = \frac{\int_0^{x'} \sigma dx'}{\int_0^{30} \sigma dx'} \quad (3)$$

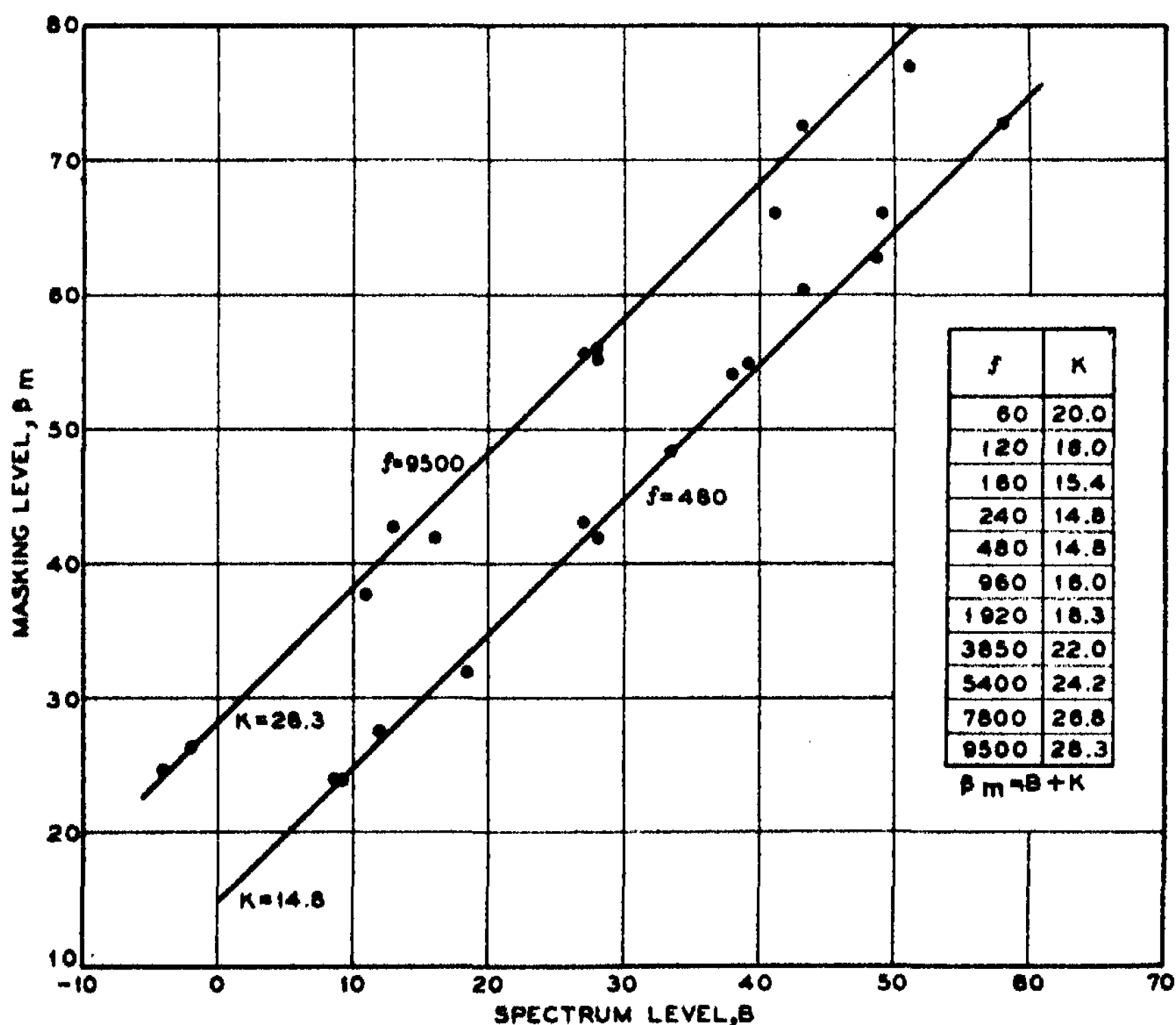


FIGURE 2

In our discussions we will use the coördinate x which can be determined directly from auditory experiments.

When a continuous noise is impressed upon the ear it produces a certain stimulation at the position x . The stimulating agent will be taken as the vibrational power acting upon the nerve endings. Let the amount of this power between positions x and $x + \Delta x$ be designated as ΔJ . Then the power J_x for one per cent of the nerve endings is given by

$$J_s = \frac{\Delta J}{\Delta x}. \quad (4)$$

The stimulation level S at any position x is defined by

$$S_x = 10 \log \frac{J_x}{J_0}, \quad (5)$$

where J_0 is the threshold stimulation. Since here we are dealing with a ratio of vibrational powers, the uncertainty of determining whether to use amplitude velocity or acceleration or any function of them as the stimulating agent does not arise. The value S_x can then be taken as the stimulation of the one per cent patch of nerve endings at the position x . This stimulating power can be spread over a small or a large patch of nerve endings and it will still produce just the threshold value. This is true because our experiments on loudness have shown that the nerve discharges near the threshold are proportional to the intensity of the sound.

Let the following experiments be performed. A broad band of thermal noise whose spectrum level B is known at each frequency is impressed upon the ear of an observer. While this noise is present, a pure tone of known frequency f is raised to the intensity I_m so that the observers just perceive its presence. The intensity level of this tone which is masked will be designated β_m and it is related to I_m by the equation

$$\beta_m = 10 \log I_m. \quad (6)$$

For a fixed frequency a set of values of β_m and B were obtained using different intensity levels of the noise.

The experimental apparatus and methods have been described elsewhere. A typical set of results is shown in figure 2 for two frequencies 9500 cycles per second and 480 cycles per second. The spectrum level B of the noise at each of these frequencies is plotted as abscissae and the intensity level of the pure tone just masked by the noise is plotted as ordinates. It was found that this relation was independent of the frequency width of the band as long as this exceeded a critical value ranging from 100 c. p. s. in the low frequencies to 1000 c. p. s. in the high frequencies. Points above $\beta_m = 80$ and below $\beta_m = 20$ are usually above the straight line which goes through the points between these levels. There is a reason for both of these departures which cannot be dealt with here. It is the straight portions of the curves which give us the information we want to use in this discussion. Since they are straight lines, equations of the form

$$\beta_m = B + K \quad (7)$$

will represent them, where K is a parameter varying with the frequency regions used. It is this variation that enables us to determine the relation

of the position of maximum stimulation x , to the frequency f of the impressed tone. Eleven frequency regions were tested and the results plotted on eleven straight lines like those of figure 2.† The resulting values of K are shown in figure 2 for the various frequencies used. The relation between the acoustic pattern and the corresponding stimulation pattern on the nerves is illustrated in figure 3. A 500-cycle band of noise between 500 and 1000 is spread into a wide stimulation pattern of 16 per cent of the nerve endings, while this same band width between 7500 and 8000 of the same acoustic intensity acts upon only one per cent of the nerve endings at position 88 as indicated. Consequently, one must raise the level of a pure tone in this high frequency region to a higher value to be perceived than if

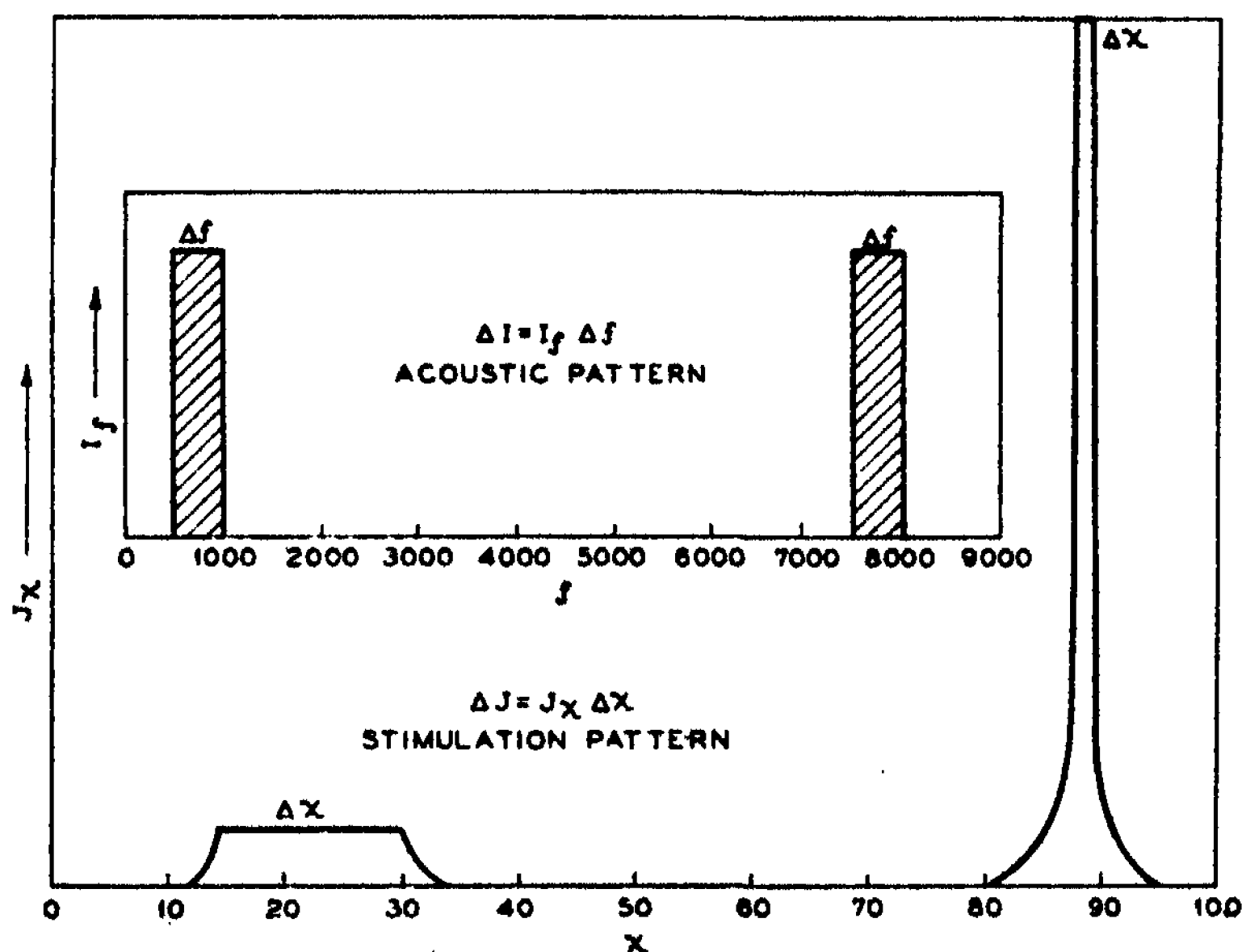


FIGURE 3

one were perceived in the low frequency region where the stimulation per nerve is not nearly so great. Conversely, if the masking level, compared to the spectrum level, is higher for the high frequency region than for the low frequency region, it shows that the frequencies are less spread out in the high and more spread out in the low frequency regions. For example, in the case illustrated the masking tone would be sixteen times greater for one than for the other.

Let us consider this relation in a quantitative way. The intensity ΔI in the thermal noise band between f and $f + \Delta f$ goes to stimulate mostly those nerve endings between x and $x + \Delta x$, where x corresponds to the position of maximum stimulation for a pure tone having a frequency f ,

and $x + \Delta x$ to the position of maximum stimulation for a pure tone having a frequency of $f + \Delta f$. In other words, the band Δf located at the frequency position f will be chosen so that the power in this particular noise band is spread over the particular patch of nerves Δx located at the position x .

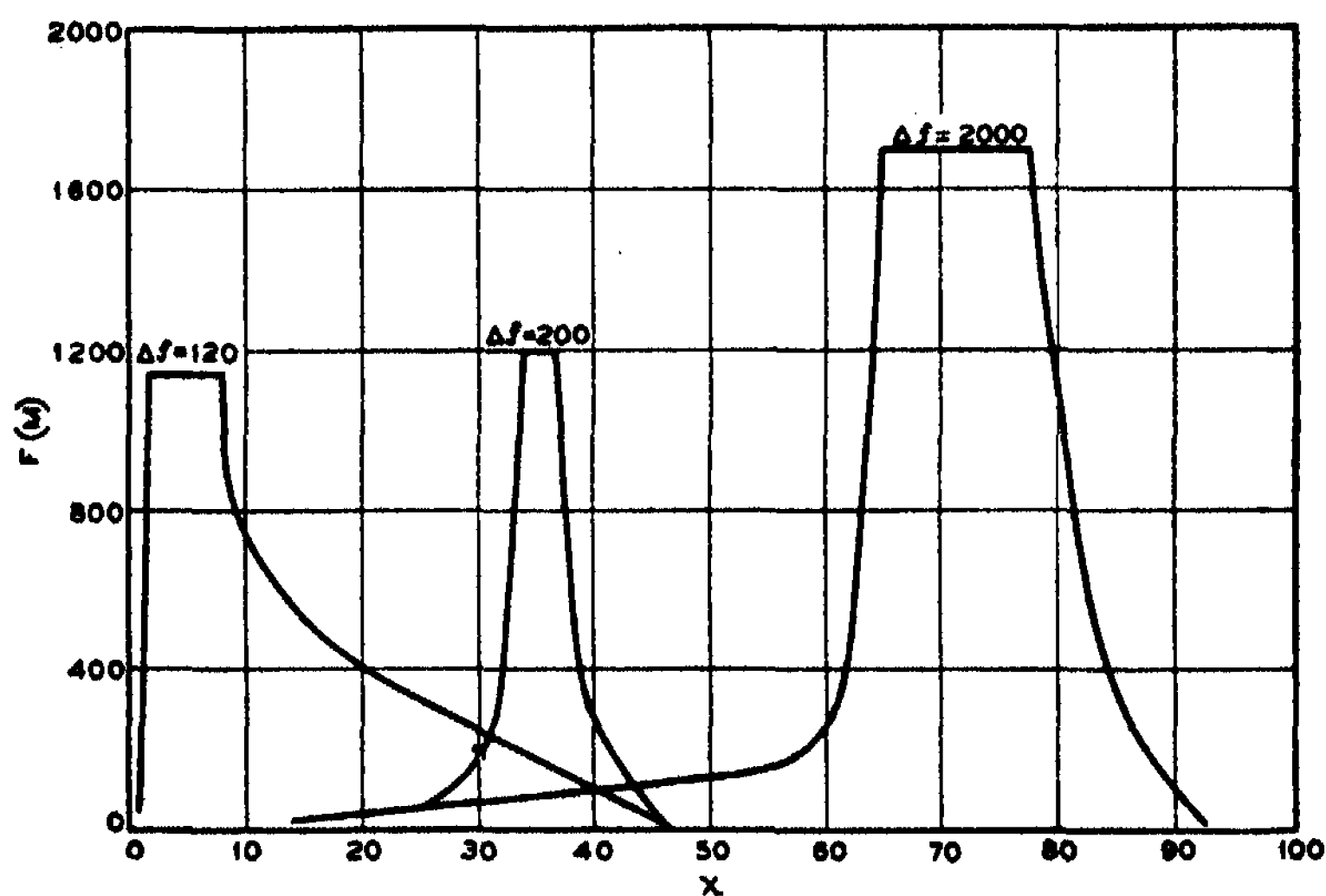


FIGURE 4

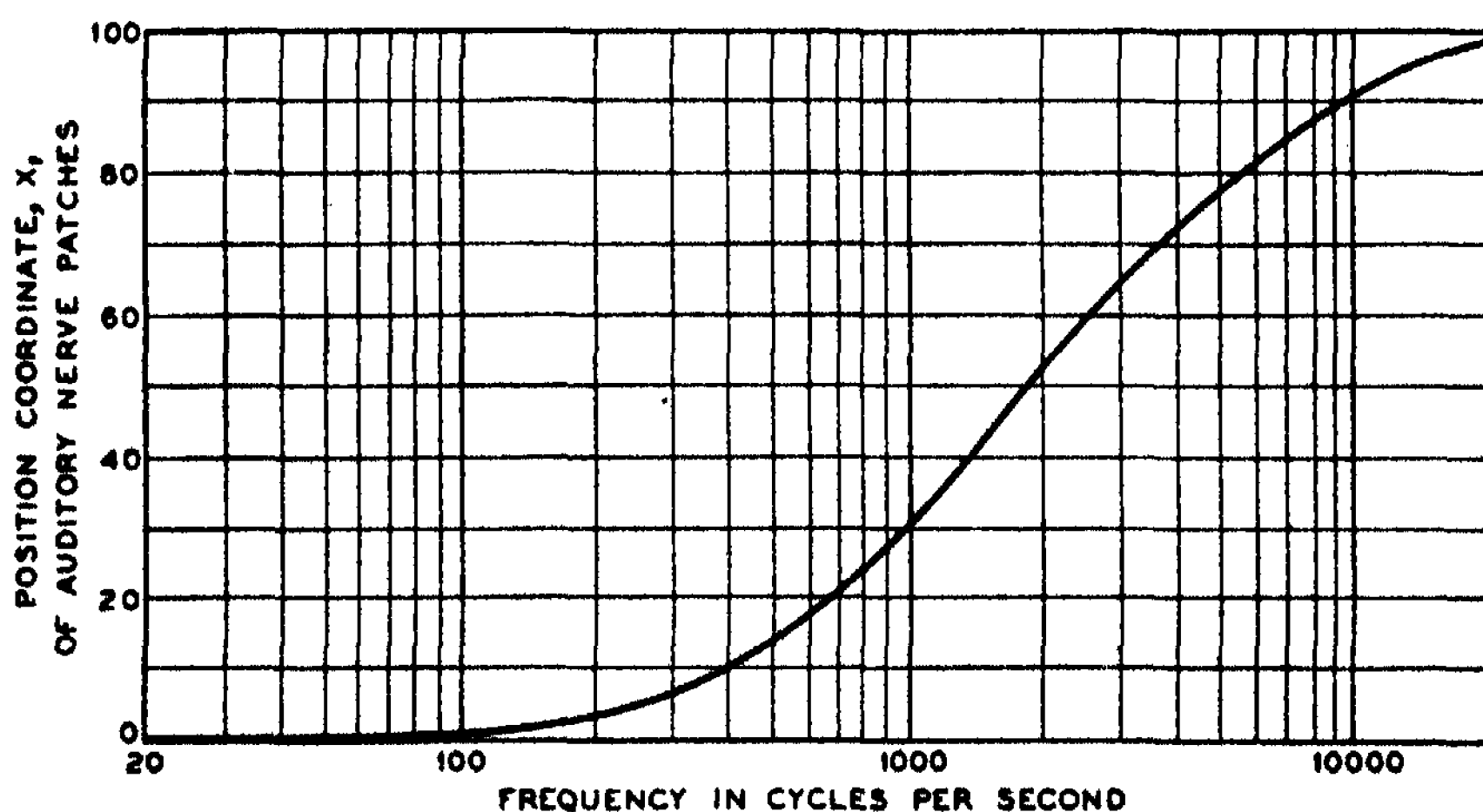


FIGURE 5

As illustrated in figure 3 the patches adjacent to Δx will also be stimulated if a single band Δf is impressed upon the ear. However, for any type of noise whose spectrum is not changing rapidly with frequency these adjacent patches will already be stimulated by the adjacent bands. For this type

of noise it is fairly accurate to assume that all the intensity in the band Δf goes to stimulate the nerve patch Δx . The response of the nerves stimulated by small bands of thermal noise has been calculated. The results of this calculation are shown in figure 4 for three different positions. It is seen that the ear is very selective with frequency and that the above assumption is a very good approximation.

Since the thermal noise band and also the masked tone are in the same frequency region, they will have approximately the same frequency discrimination produced by the electro-acoustical system generating the sounds and also by the transmission system in the ear. The acoustical intensity ΔI

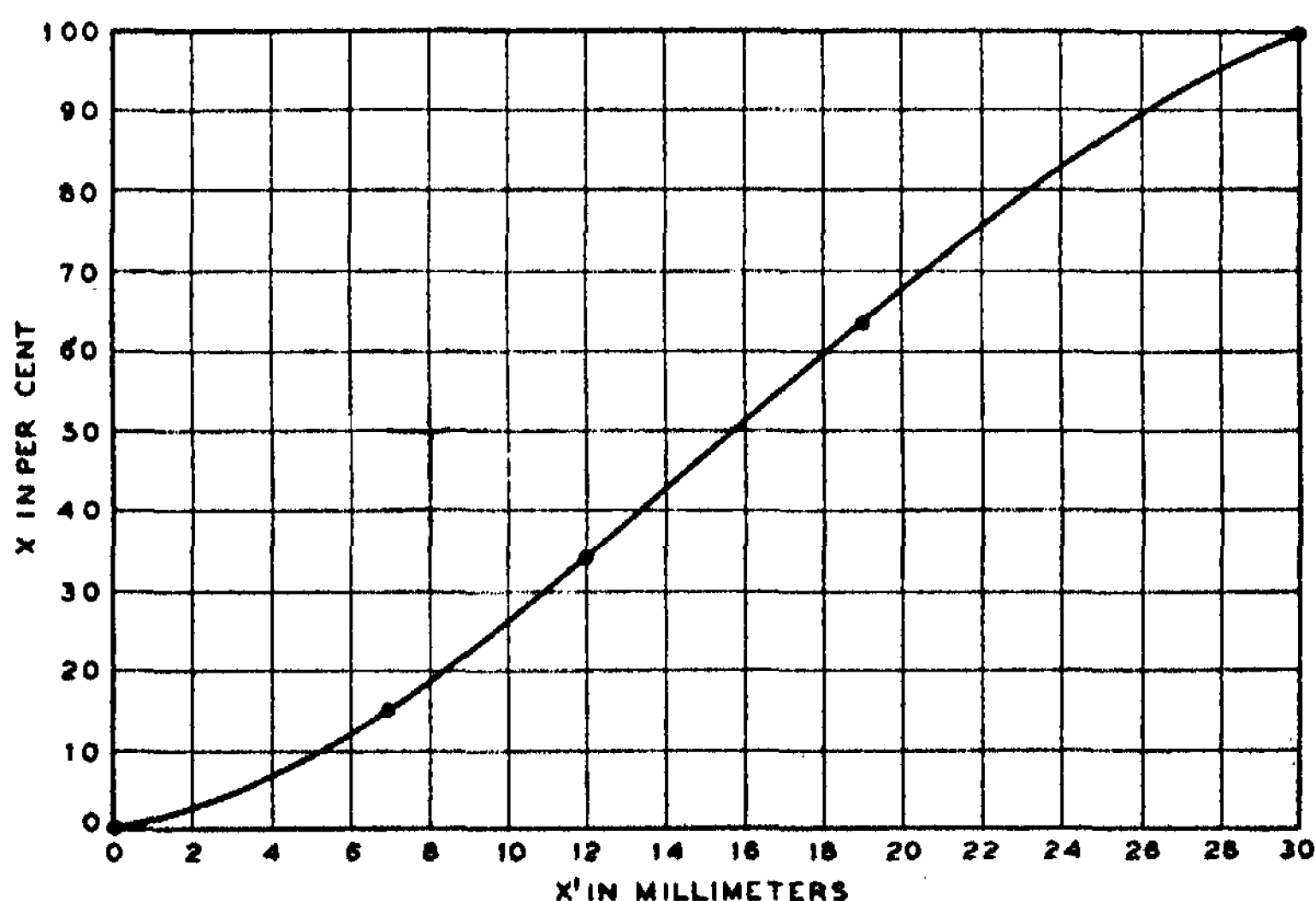


FIGURE 6

produces the stimulation ΔJ . The acoustical intensity I_m produces the stimulation J_m at the same nerve position x as for ΔJ . Therefore in the range where the system is linear

$$\frac{\Delta I}{\Delta J} = \frac{I_m}{J_m} = \frac{I_f \Delta f}{J_x \Delta x}. \quad (8)$$

If we consider Δf and Δx as differentials and integrate we have

$$x = \int_0^f \frac{I_f J_m}{I_m J_x} df. \quad (9)$$

It is now assumed that $\frac{J_m}{J_x}$ is a constant C independent of position x and consequently of the frequency f .

$$C = \frac{J_m}{J_x}. \quad (10)$$

Also, equation (9) must fulfil the condition that $x = 100$ when $f = \infty$. If these conditions are applied and the values of B and β_m from equations (2), (6) and (7), then equation (9) reduces to

$$x = C \int_0^f \frac{I_f}{I_m} df = C \int_0^f 10^{-K/10} df \quad (11)$$

where

$$C = \frac{100}{\int_0^\infty \frac{I_f}{I_m} df} = \frac{100}{\int_0^\infty 10^{-K/10} df}. \quad (12)$$

So we can substitute the values of K from figure 2 and integrate these equations graphically.

In the region where the system is linear

$$\frac{I_m}{I_0} = \frac{J_m}{J_0} \quad (13)$$

where I_0 is the threshold intensity of a band of thermal noise of small width located at the frequency position f corresponding to that of the tone which is masked, and J_0 the stimulation necessary for threshold at the position x corresponding to f . If we multiply the numerator and denominator of the left-hand side of this equation by J_x and then reduce the equation to decibels by taking $10 \log$ of both sides, there results

$$\beta_m - \beta_0 = S + 10 \log \frac{J_m}{J_x} = S + 10 \log C. \quad (14)$$

The left-hand side of this equation is, by definition, the masking M . This shows that for a linear system the assumption that J_m/J_x is a constant is equivalent to assuming that for a constant masking there is a constant stimulation. In other words, the amount of masking M is a measure of the stimulation S and the difference between these two quantities is simply $10 \log$ of the constant given by equation (10). If we express equation (7) in decibels and then rearrange the terms, there results

$$\beta_m = B + 10 \log C + 10 \log \frac{df}{dx}. \quad (15)$$

From this equation it is seen that the intensity level β_m of the masked tone can be calculated from the spectrum level B of the noise provided the relation between x and f is known.

Using the values of K shown in figure 3, equations (11) and (12) were integrated graphically. The value for C was found to be 1.1, or within the experimental error equal to unity. This means that when the stimulation J_m due to the tone being masked is equal to the stimulation J_z upon one per cent of the nerves then it will just be perceived by a typical observer. Also, this indicates that the masking M is directly equal to the stimulation S and may be considered a means of measuring it. The resulting values of f and x obtained from equations (11) and (12) are shown in the curve of figure 5. The relationship between f and x indicates that frequencies below about 150 cycles per second all have the position of maximum stimulation on the first one per cent patch of nerve endings. When the frequency of the stimulating tone reaches 1000 cycles per second the position of maxi-

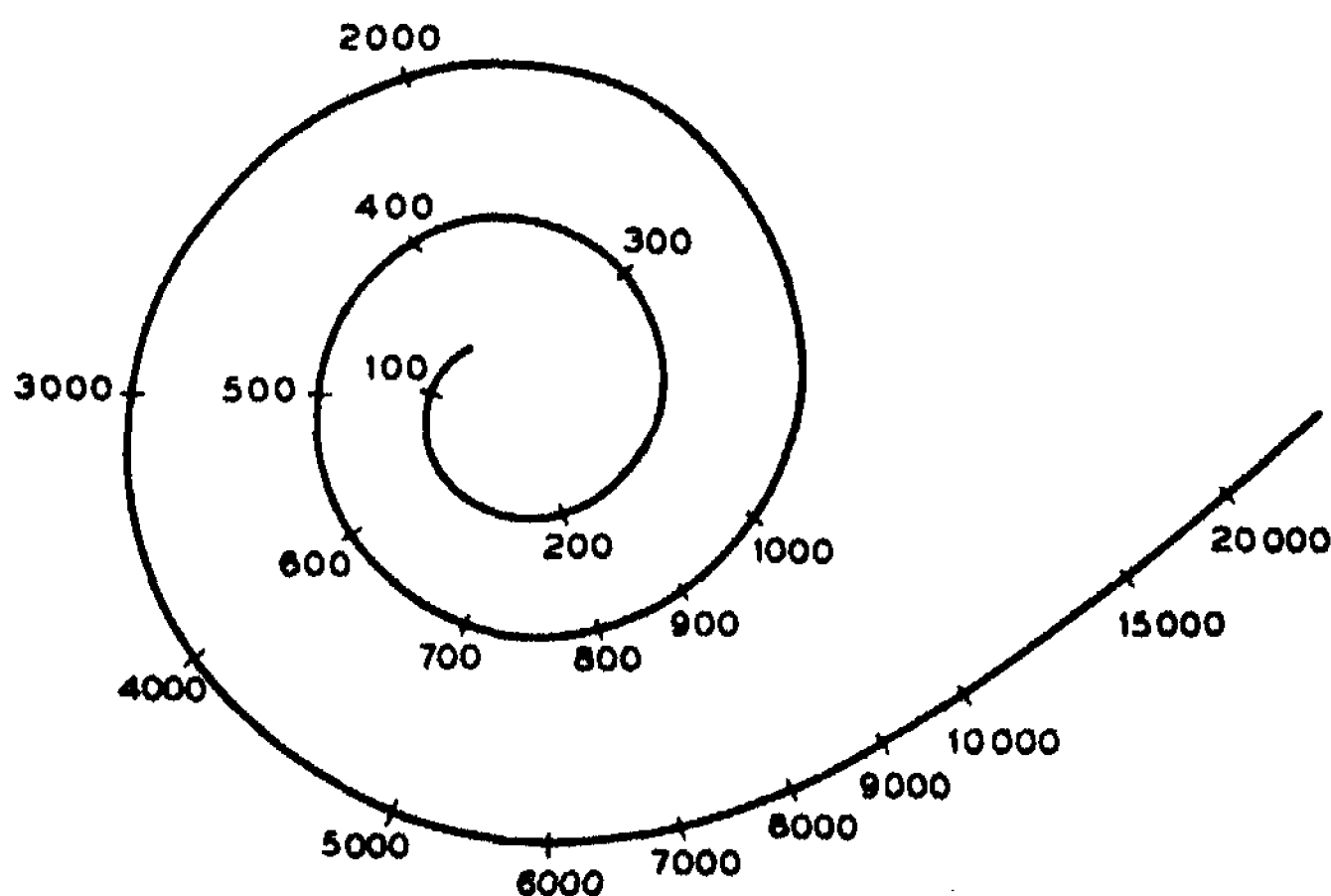


FIGURE 7

mum stimulation has passed over approximately 30 per cent of the nerve endings.

It will be remembered that the values of x refer to nerve patches in such a way that for equal intervals Δx there are an equal number of nerve endings. If the nerve endings are distributed uniformly along the basilar membrane then the values of x used in this discussion will correspond to one per cent of the total length of the basilar membrane. If, however, the nerve endings are not distributed uniformly, then the length dx must be stretched or contracted depending upon the nerve density being less or greater than the average. Let x' be the distance from the helicotrema in millimeters.

Guild† has made measurements of the number of ganglion cells along the basilar membrane. He divided the cochlea into four parts, namely (1) upper middle and apical, (2) lower middle, (3) upper basal, (4) lower basal.

He made a count of the total number of ganglion cells in each of these parts. They are given below in column 3 of table 1. Steinberg has esti-

TABLE 1			
POSITIONS	x'	COUNT	x
Upper Middle plus apical	0-7	4,200	0-14.5
Lower Middle	7-12	5,600	14.5-33.8
Upper Basal	12-19	8,500	33.8-63.1
Lower Basal	19-30	10,700	63.1-100
Total	0-30	29,000	0-100

mated the distance in millimeters along the basilar membrane for each of these parts to be that given in the column under x' . The corresponding values of x are then easily calculated as the per cent of the total cells up to the position corresponding to x' . They are given in the last column.

It is seen that these data on the number of ganglion cells allow us to calculate only three points besides the two end-points. These values of x and x' are plotted on figure 6. A smooth curve passed through these points. Using this relation, then the values of x and f from figure 5 can be transferred to values of x' and f . In figure 7 this relation is plotted on a spiral having the dimensions shown in table 1. The positions given in this spiral agree with previous determinations within the accuracy of those determinations.

* All intensity values used in this paper are expressed by this same unit.
† I am indebted to Mr. W. A. Munson for doing this experimental work.
‡ Guild, Stacy R., *Acta Oto-Laryng.*, 17, 207-245 (1932).

THE DIMENSIONLESS CONSTANTS OF PHYSICS

BY ARTHUR E. HAAS

UNIVERSITY OF NOTRE DAME

Communicated June 8, 1938

Physics appears to be characterized by several universal constants which are pure numbers. One is the ratio of the mass of the proton to the mass of the electron, i.e., $m_p/m = 1835$, another the ratio $e^2/(hc)$, where e is the fundamental charge, h Planck's constant and c the velocity of light. A third is the ratio $e^2/(G m_p m)$, where G represents the gravitational constant. An important fourth number, under the assumption of a finite uni-

verse, is given by the ratio M/m_p , where M denotes the total mass of the universe. It appears that a fifth constant, the ratio of the total number of neutrons to the total number of protons, should be introduced, this ratio being referred either to a static universe, or under the assumption of an expanding universe, to its initial equilibrium state.

It will be the task of this consideration to derive the result that the empirical constants of atomic physics yield for this ratio a value equal to unity to a high degree of approximation, provided that we can accept as correct the results of two earlier papers based on wave-mechanical deductions and published by Eddington and by Sitte and Glaser, respectively.

Eddington,¹ in 1931, derived the simple relation

$$R^2 = N_e a^2, \quad (1)$$

where R denotes the equilibrium radius of the universe, N_e the total number of electrons and a ($= e^2/(mc^2)$) the classical radius of the electron.

Sitte and Glaser² arrived at a formula which may be written in the following form

$$h/(M c) = R N_p^{-1/2} (N_p + N_e)^{-1}, \quad (2)$$

where N_p is the total number of protons; the existence of neutrons was not taken into account by Sitte and Glaser. Since, however, the only properties of protons and electrons which play a rôle in Sitte and Glaser's considerations are that the mass of the electron is insignificant in comparison with the mass of the proton, and that the universe as a whole is electrically neutral, equation (2) may be generalized as follows:

$$h/(M c) = R N^{-1} N'^{-1/2}, \quad (3)$$

where N is the total number of primordial particles of which N' are heavy in the sense that the mass of the other ($N - N'$) particles may be disregarded in comparison with their mass.³

If we denote the number of neutrons by N_n and remember that N_e equals N_p , we have

$$N = N_n + 2N_p, \quad N' = N_n + N_p, \quad M = (N_n + N_p) m_p. \quad (4)$$

If we put

$$N_n = x N_p \quad (5)$$

we thus find

$$h/(m_p c) = R N_p^{-1/2} (1 + x)^{1/2} (2 + x)^{-1}. \quad (6)$$

By combining this relation with equation (1) and remembering the equality of N_p and N_n , we obtain

$$(2 + x)(1 + x)^{-1/2} = (m_p/m) \times e^2/(hc). \quad (7)$$

The empirical value of the right-hand side is 2.13 and the solution of equation (7) is a value of x slightly above unity. It would just be equal to one if we could put the right-hand side of equation (7) equal to 2.12, thus diminishing its empirical value by half a per cent.

Thus, if the equations derived by Eddington and by Sitte and Glaser hold, we arrive at the conclusion that in the state of equilibrium the number of neutrons nearly equals the number of protons. On the other hand, a relation, first pointed out by the author,⁴ which connects purely atomic constants and which is fulfilled to within an error of about half a per cent, i.e.,

$$m_p/m \times e^2/(hc) = 3/\sqrt{2} \quad (8)$$

may be derived from the formulae of Eddington and Sitte and Glaser by assuming the equality of the numbers of neutrons and protons in the equilibrium state.

¹ A. S. Eddington, *Proc. Roy. Soc. London*, A135, 605 (1931).

² K. Sitte and E. Glaser, *Zeitschr. Physik*, 88, 103 (1934).

³ Concerning the details, cf. the paper by Sitte and Glaser.

⁴ A. E. Haas, *Science*, June (1938): The classical radius of the electron is related to the Compton wave-length of the proton as 3 to $\sqrt{2}$.

ON THE RELATIVE CONTRIBUTIONS OF NATURE AND NURTURE TO AVERAGE GROUP DIFFERENCES IN INTELLIGENCE

BY BARBARA S. BURKS

CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR

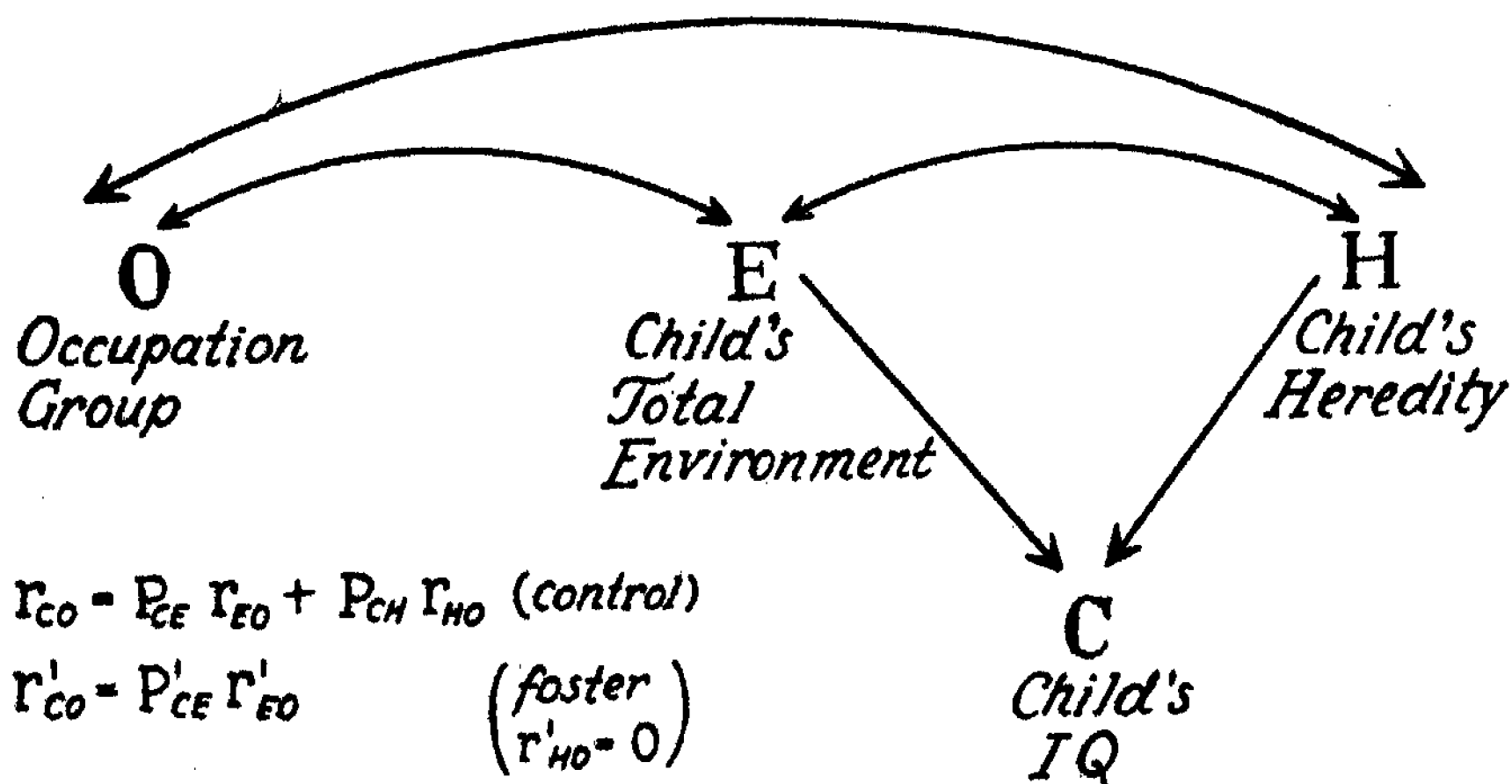
Communicated June 13, 1938

It has become a more or less accepted commonplace that environmental differences, although having a demonstrable influence upon individual differences in IQ, are less potent than natural differences under the conditions ordinarily met in our urban culture. No wholly satisfactory quantitative estimate of the relative rôles of nature and nurture has yet been possible, but various estimates agree in placing the contribution of nurture under 50 per cent, and probably considerably under. (See references 1 to 5.)*

Far less satisfactory is the present position of the problem of average socio-economic *group* differences in intelligence—in fact it is only in the last few years that it has been generally recognized and discussed as a separate problem requiring its own techniques for solution. There is no simple correspondence between the contributions of nature and nurture to group and individual differences, but the same types of data are crucial for both problems. The writer has drawn upon two sources of data applicable to the problem of average group differences in IQ with respect to father's occupation—her previous study conducted at Stanford University of the IQ's of foster children and "own" children in relation to parental intelligence and home background, and the more recent study by Leahy at University of Minnesota dealing with the same type of material.

The comparisons involved are unusually straightforward, for it can be shown that the difference in mean IQ of "own" children grouped according to father's occupation is composed of two additive heredity and environment factors, the latter being given by the corresponding difference in group means of foster children. Wright has kindly furnished a proof, which depends upon the use of path coefficients, as follows:

Child's IQ can be represented as completely determined by the two factors—heredity (i.e., the *child's* genetic constitution) and total environment, which may be (and undoubtedly are) more or less correlated with each other. Occupational status of father is clearly correlated with the child's total environment. The increased differences in the control data indicate that it is also correlated with the child's heredity in the controls. The reasons (involving father's intelligence) need not be represented. The following diagram represents these essential points:



The observed differences in IQ in relation to given differences in occupation group are of the nature of regression coefficients.

$$b_{CO} = r_{CO} \frac{\sigma_c}{\sigma_o} = (p_{CE}r_{EO} + p_{CH}r_{HO}) \frac{\sigma_c}{\sigma_o} \text{ (control),}$$

$$b'_{CO} = r'_{CO} \frac{\sigma'_c}{\sigma'_o} = (p'_{CE}r'_{EO}) \frac{\sigma'_c}{\sigma'_o} \text{ (foster).}$$

The partial regression coefficients measuring the concrete effects of H and E on C should be the same in foster and control data in spite of differences in correlations, path coefficients or standard deviations.

$$p_{CE} \frac{\sigma_c}{\sigma_o} = p'_{CE} \frac{\sigma'_c}{\sigma'_o}, \quad p_{CH} \frac{\sigma_c}{\sigma_o} = p'_{CH} \frac{\sigma'_c}{\sigma'_o}.$$

If now we assume that the correlation between total environment and occupation group is the same in the two bodies of data but that there is no correlation between child's heredity and occupation group in the foster data (no effective selection of children):

$$r_{EO} = r'_{EO}, \quad r'_{HO} = 0,$$

$$b_{CO} = p_{CE}r_{EO} \frac{\sigma_c}{\sigma_o} + p_{CH}r_{HO} \frac{\sigma_c}{\sigma_o} \text{ (control),}$$

where $p_{CE}r_{EO} \frac{\sigma_c}{\sigma_o} = b'_{CO}$ given by the foster data.

Thus the difference in the controls can properly be analyzed into two additive portions tracing through the correlation of occupation group with child's environment, which is the same as the total difference in the foster data, and tracing through the correlation with child's heredity.

The published Minnesota study provides the needed tabulations for occupational group comparisons. It was necessary to go back to the original data of the Stanford study and tabulate them according to a similar scheme.[†]

The results are shown in tabular form. Table 1 contains the group means and dispersions of intelligence test scores; those of the parents as well as of the children are included by way of additional interest. Table 2 contains derived estimates of the relative contributions of nature and nurture to occupational group differences. The "nurture" column of table 2 is simply the ratio (in per cent) of group difference in foster children to group difference in control "own" children. The "nature" column is 100 minus this ratio, or the ratio of increment of control difference to control difference. In table 3 are presented estimates of the relative contribu-

tions of nature and nurture based upon the total data of the Stanford and Minnesota studies, singly and in combination. Sampling errors of these estimates are also included.

Two methods, giving virtually the same results, were used for obtaining combined estimates of the nature and nurture contributions (table 3).

1. The occupational group differences shown in table 2 were summed for the control and foster groups separately, and the ratio of the foster sum to the control sum was computed. This procedure is sufficiently ac-

TABLE 1
MEANS AND DISPERSIONS OF INTELLIGENCE SCORES BY OCCUPATIONAL GROUP

	STANFORD STUDY OCCUPATION OF FATHER (OR FOSTER FATHER)				MINNESOTA STUDY OCCUPATION OF FATHER (OR FOSTER FATHER)				
	PRO- FESS. I.	HIGHER BUS., SEMI- PROP. II.	LOWER BUS. III.	SKILLED LABOR IV.	PROFESS. I.	BUS. MGR. II.	SKILLED TRADES & CLERI- CAL III.	SEMI- SKILLED IV.	BLT. SKILLED & DAY LABOR V.
Foster children									
Mean (IQ)	109.1	108.6	108.0	104.6	112.6	111.6	110.6	109.4	107.8
S. D. (IQ)	17.2	14.5	14.3	16.7	11.8	10.9	14.2	11.8	13.6
No.	32.0	47.0	41.0	43.0	43.0	38.0	44.0	45.0	24.0
Control children									
Mean (IQ)	118.7	118.5	115.5	106.1	118.6	117.6	106.9	101.1	102.1
S. D. (IQ)	15.4	12.2	18.6	12.4	12.6	15.6	14.3	12.5	11.0
No.	18.0	33.0	27.0	18.0	40.0	42.0	43.0	46.0	23.0
Foster parents*									
Mean	221.8	207.3	201.2	184.7	59.6	59.6	49.6	39.7	38.4
S. D.	22.6	30.8	29.7	30.3	8.0	6.7	11.9	12.3	11.2
No.	24.0	40.0	34.0	34.0
Control parents*									
Mean	221.6	221.8	192.0	176.2	64.6	57.1	51.8	44.0	38.3
S. D.	24.4	30.4	33.4	31.6	5.4	10.0	11.5	11.5	9.0
No.	18.0	32.0	27.0	18.0

* In the case of the Stanford study, data are for Stanford-Binet mental age in months of foster fathers and control fathers. In the case of the Minnesota study, data are for mid-foster parent and mid-parent point score on the Otis Test of Mental Ability.

curate for an over-all appraisal when the population numbers and standard deviations of the sub-groups do not differ in an extreme manner. The sampling errors of the estimates thus obtained, however, are difficult to determine because of the lack of independence of the group differences entering the sums.

2. Using the relation,†

$$\frac{x_F - \bar{x}_F}{\sigma_F} = r \frac{x_C - \bar{x}_C}{\sigma_C}$$

(1)

and assuming a true r of unity (i.e., a constant proportional effect upon IQ of nurture and nature in the small range of IQ levels with which we are concerned), we may estimate the proportional contributions by the ratio**

$$R = \frac{\sigma_F}{\sigma_C}.$$

(2)

The sampling variance of R is found by squaring and averaging logarithmic differentials, viz.,

$$V_o(R) = R^2 \left[\frac{V_o(\sigma_F)}{\sigma_F^2} + \frac{V_o(\sigma_C)}{\sigma_C^2} \right].$$

(3)

σ_F , σ_C represent the dispersions of sets of scores in which each score is drawn from a unique universe of given central tendency and sampling

TABLE 2

ESTIMATES OF THE RELATIVE CONTRIBUTION OF NATURE AND NURTURE TO DIFFERENCES IN MEAN INTELLIGENCE SCORE OF CHILDREN GROUPED ACCORDING TO FATHER'S OCCUPATION

GROUPS COM- PARED	STANFORD STUDY					MINNESOTA STUDY				
	CONTROL	FOSTER	CONTROL CONTRIB. CONTRIB. CONTRIB.			CONTROL	FOSTER	CONTROL CONTRIB. CONTRIB. CONTRIB.		
			MINUS	OF	OF			MINUS	OF	OF
			FOSTER	NURTURE	NATURE			FOSTER	NURTURE	NATURE
I-II	0.2	0.5	1.0	1.0
I-III	3.2	1.1	2.1	34.4	65.6	11.7	2.0	9.7	17.1	82.9
I-IV	12.6	4.5	8.1	35.8	64.2	17.5	3.2	14.3	18.3	81.7
I-V	16.5	4.8	11.7	29.1	70.9
II-III	3.0	0.6	2.4	20.0	80.0	10.7	1.0	9.7	9.3	90.7
II-IV	12.4	4.0	8.4	32.3	67.7	16.5	2.2	14.3	13.3	86.7
II-V	15.5	3.8	11.7	24.5	75.5
III-IV	9.4	3.4	6.0	36.2	63.8	5.8	1.2	4.6	21.7	79.3
III-V	4.8	2.8	2.0	58.4	41.6
IV-V	-1.0	1.6

fluctuation. Hence their sampling variances (to be used in formula 3) cannot be calculated by the usual formula. A formula for $V_o(\sigma_F)$, $V_o(\sigma_C)$ may be derived as follows:

$$\sigma^2 = S \frac{(x_i - \bar{x})^2}{n - 1}$$

(4)

$$= \frac{(x_1 - \bar{x})^2 + \dots + (x_n - \bar{x})^2}{n - 1}.$$

Taking differentials, squaring and averaging,

$$V_o(\sigma) = S \frac{[(x_i - \bar{x})^2 (V_o(x_i) + V_o(\bar{x}) - 2/n V_o(x_i))]}{\sigma^2(n - 1)^2}$$

[$V_o(x_i) = \sigma^2 x_i / n$, obtainable from table 1].

The difference in estimated contributions of nature and nurture yielded by the Stanford study ($<2/3, >1/3$) and the Minnesota study ($>3/4, <1/4$) may be due to sampling error alone, although examination of the slope of the regression of foster means upon control means in the two studies suggests that the estimate of the potency of nurture is rather consistently lower in the Minnesota study than in the Stanford study. This may perhaps be accounted for by the fact that there was less opportunity for "selective placement" in the Minnesota foster children, who were limited to those placed in adoptive homes under 6 months of age, than in the California foster children who were placed up to 12 months of age.

TABLE 3

ESTIMATES OF THE RELATIVE CONTRIBUTION OF NATURE AND NURTURE BASED UPON
TOTAL DATA, WITH TESTS OF SIGNIFICANCE

	STAN- FORD STUDY	MINNE- SOTA STUDY
Unweighted sum of control group differences	40.8	99.0
Unweighted sum of foster group differences	14.1	23.6
Estimated contribution of nurture $\left(\frac{\text{foster grp. diff.}}{\text{control grp. diff.}}\right)$	0.345	0.238
Estimated contribution of nature (1-nurture contrib.)	0.655	0.762
Dispersion of control group means (σ_C)	5.92	8.37
Dispersion of foster group means (σ_F)	2.03	1.87
Estimated contribution of nurture ($R = \sigma_F/\sigma_C$)	0.344	0.224
Estimated contribution of nature ($1 - R$)	0.656	0.776
Sampling variance of R ($V_o(R)$)	0.054	0.017
Sampling error of R ($\sqrt{V_o(R)}$)	0.232	0.131
Information ($1/V_o(R)$)	18.59	58.82
Combined \bar{R} (weighted according to information)	0.253	
$1 - \bar{R}$	0.747	
$V_o(\bar{R})$	0.013	
Sampling error ($\sqrt{V_o(\bar{R})}$)	0.114	

Combining the estimates in the two studies according to the amount of information yielded by each (inversely as their sampling variances), we arrive at an estimated $3/4, 1/4$ as the relative contributions of nature and nurture, with approximately even chances that the contribution of nurture is in truth between 18 and 33 per cent.

Finally, it may be pointed out that differences in mean intelligence scores of the parents according to occupational group might be expected to derive from natural differences in a higher proportion than do those of their offspring, since intelligence is one of the components that enters directly into

the selection of an occupation, or the selection of a person by an occupation. This truism is borne out by the higher regression of intelligence on occupation in the parent groups than in the offspring groups.

* Reference 3 contains quantitative estimates of the contributions of nature and nurture which have been strongly criticized. However, the simple correlation, 0.67, between the intelligence scores of identical twins reared apart—which would rise even higher if corrected for attenuation, and for the restricted range of talent—is in itself weighty evidence for the predominant influence of natural inheritance.

† To avoid the slightest possibility of bias, the classification of fathers' occupations from the Stanford data was made "blind," i.e., with no knowledge as to the intelligence of the fathers, mothers, their children or foster children.

‡ In the formulae which follow, \bar{x} , $\bar{\bar{x}}$ and σ refer to the occupational group means, the mean of the means and the standard deviation of the means, respectively.

** This formula neglects a small error factor involving the magnitude of the obtained σ 's in relation to the true σ 's [i.e., $\sigma_{\infty}^2 = \sigma^2(r)$, where r is a reliability coefficient].

¹ Burks, B. S., *27th Yearbook Nat. Soc. Stud. Educ.*, Part I, 219–316 (1928).

² Leahy, A. M., *Genet. Psychol. Monog.*, 17, 235–308 (1935).

³ Newman, H. H., Freeman, F. N., and Holzinger, K. J., *Twins*, Univ. Chicago Press, pp. xvi + 369 (1937).

⁴ Willoughby, R. R., *27th Yearbook Nat. Soc. Stud. Educ.*, Part I, 55–59 (1928).

⁵ Wright, S., *Jour. Amer. Stat. Assoc. Suppl.*, 26, 155–163 (1931).

A METAGALACTIC DENSITY GRADIENT

BY HARLOW SHAPLEY

HARVARD COLLEGE OBSERVATORY

Communicated June 14, 1938

1. In a discussion of the evidence bearing on the isotropic distribution of galaxies it was shown four years ago that at a distance of about 10^8 light-years to the north of the galactic plane the density is thirty or more per cent greater than at the same distance to the south.¹ Several different ways of discussing the extensive observational material yielded the higher density in the north. Similar inequalities were found to be present also at shorter distances, but beyond 10^8 light-years both the Harvard and the Mount Wilson data suggest tentatively the disappearance of the north-south inequality.

Quantitatively the north-south difference is not closely determined by the early work because the nebular counts were made in relatively small sample areas; but the existence of the difference is unquestionable, as is also the occurrence of non-uniformity in nebular distribution arising from the metagalactic clouds.² These well-established large-scale inequalities have been pretty generally ignored in discussions of the expanding uni-

verse; the assumptions of isotropy and large-scale uniformity have persisted, leading of late to somewhat remarkable deductions.

From surveys with reflecting telescopes Hubble has found a radial density gradient which, over an interval of 2.5×10^8 light-years, amounts to a density change of something less than twenty per cent.³ To eradicate this ap-

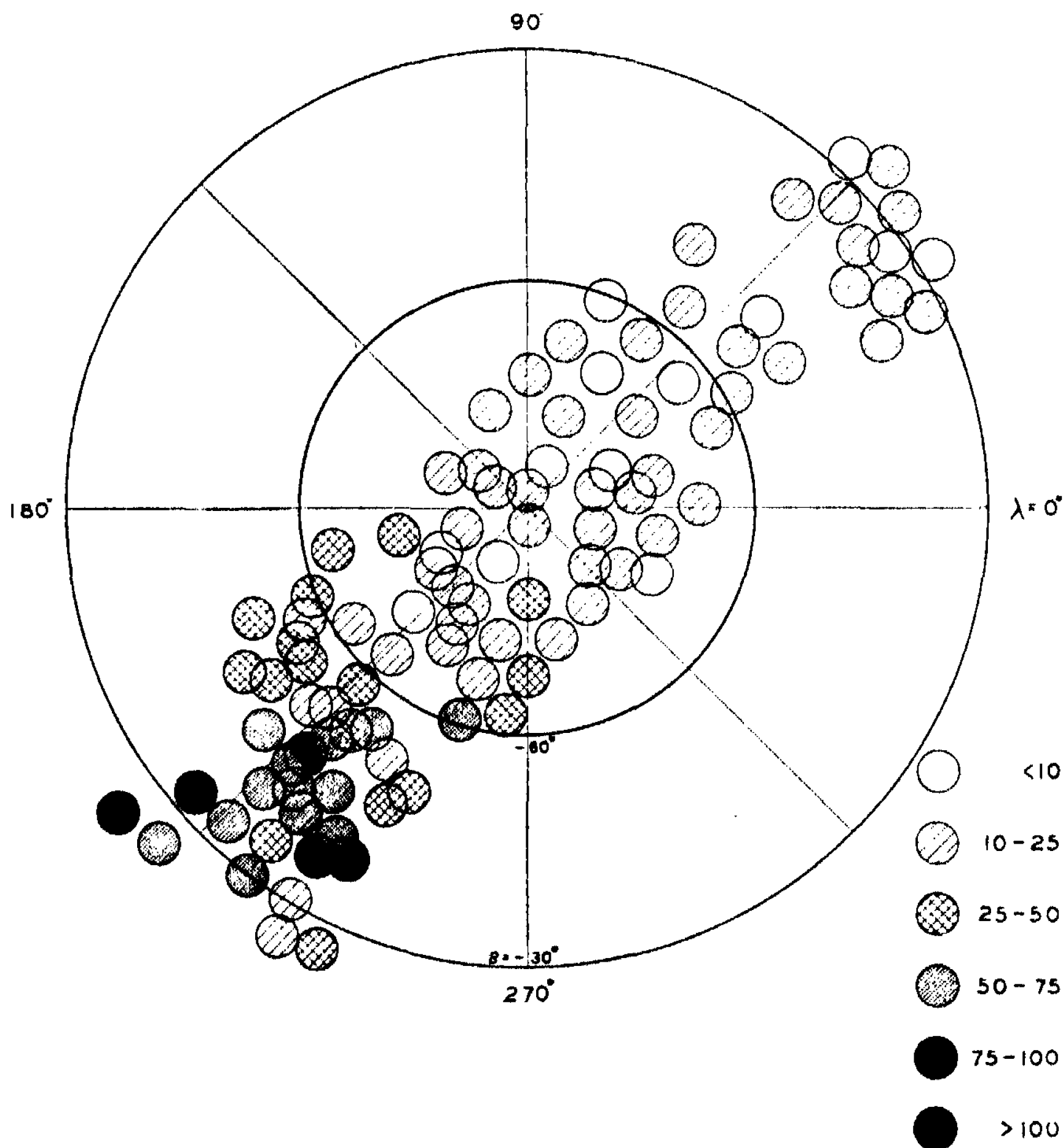


FIGURE 1

The density distribution in a thirty-degree zone across the south galactic pole.

parent increase in density with distance and restore nebular distribution to essential uniformity, he suggests the abandonment of the interpretation of red-shift as velocity of recession and the abandonment, therefore, of the hypothesis of the expanding universe, introducing in its place some new principle to account for the observed red-shift. But the evidence for

disappears, according to Hubble,³ if the scale is too restricted by 0.12 between magnitudes 18.5 and 21.0. The north-south inequalities found from the Harvard surveys four years ago involve the inter-comparison of frequencies in opposite regions, and such nebular counts are also moderately free of the effects of scale errors in the magnitude system.

3. The work on the south galactic cap (galactic latitudes -55° to -90°) has now been extended in order to explore further the progressive though somewhat irregular increase of nebular frequency as the survey crosses the south galactic pole from the northwest to the southeast. The increase was grossly shown when we found in the four quadrants the following numbers of galaxies,* to the average magnitude 18.2 (number of plates in parentheses):

Northwest	10,800 (18)	Southwest	22,800 (21)
Northeast	19,000 (18)	Southeast	23,000 (23)

Reducing the counts to a common magnitude and using only results from the inner nine square degrees on each plate, we obtain the following values for the number per square degree:

Northwest	31.87	Southwest	51.10
Northeast	54.17	Southeast	66.54

The richest quadrant, on the average, is twice as rich as the poorest; the transverse distance involved is of the order of 10^8 light years.

4. The manner in which the survey has been extended is most clearly indicated by the polar diagram in figure 1. The south galactic pole is at the center; galactic latitudes and longitudes form the coördinate system. In a zone thirty degrees wide across the south galactic polar cap, centrally aligned on the longitude circle 45° , 225° , the positions are plotted of one hundred survey plates (each of three hours' exposure with the Bruce telescope). The frequency of galaxies is roughly indicated by the shading. The area of each circle is approximately twenty-five square degrees—the same as that covered effectively on the sky by a Bruce plate.

The diagram shows that the density continues to increase to the southeast, beyond the limits of the earlier survey.⁴ Whether the maximum has been reached at latitude -35° (right ascension 5^h , declination -60°), before the absorption of the Milky Way system makes itself appreciably felt, cannot yet be determined. The observed falling-off in density beyond the maximum is probably a feature of metagalactic structure in this region, but may be also in part caused by outlying Milky Way absorption, which has

* The objects lying outside the twenty-five square degrees on each of the eighty plates are not included. They approximately balance in number the objects appearing twice in overlapping of plates for which correction has not been made in the summation above.

been shown in a study of the south equatorial polar cap to be widespread in this part of the sky.²

In table 1 mean values, reduced to the convenient magnitude limit 17.9, are given for the results from the central nine square degrees of the plates, and also for the surrounding sixteen square degrees. Each mean includes the results of five plates, and therefore forty-five square degrees and eighty square degrees, respectively, are represented by each entry and by the plotted points of figures 2 and 3. The last column of the table gives for each group of five plates the total number of galaxies actually observed within the twenty-five square degrees.

TABLE 1

POSITIONS AND AVERAGE NUMBERS OF GALAXIES PER SQUARE DEGREE

GALACTIC		LOGARITHMS		TOTAL NUMBER OF GALAXIES
LONG.	LAT.	\bar{N}_{45}	\bar{N}_{80}	
45°	-29°	1.14	0.88	3,630
	-36	1.10	0.85	4,353
	-47	1.18	1.00	3,788
	-61	0.99	0.87	2,773
	-68.5	1.07	0.92	3,475
	-76.5	1.12	0.90	3,440
	-81	1.13	0.91	5,992
	-87	1.21	1.08	4,789
	-88	1.08	0.88	4,570
225	-81	1.20	0.95	4,648
	-75.5	1.30	1.10	5,650
	-71	1.30	1.08	5,846
	-68.5	1.50	1.29	7,582
	-58	1.45	1.35	4,363
	-54	1.55	1.38	4,820
	-50.5	1.60	1.47	4,918
	-45.5	1.76	1.58	6,669
	-41.5	1.90	1.71	6,557
	-35	1.91	1.70	5,706
	-28	1.66	1.48	3,978
Total				97,547

So much area and so many objects are included in each mean that they no doubt smooth out large local irregularities. But the plots are given here chiefly to show the large-scale non-uniformity that stretches over 125 degrees of the southern sky. The completion of the eighteenth magnitude survey for the southern hemisphere will permit more detailed description of the nebular distribution; but as it stands, we see a conspicuous and steady rise in the frequency from $\bar{N}_{45} = 10$, at about latitude -60° in the northwest quadrant, to $\bar{N}_{45} = 80$, at about latitude -35° in the southeast quadrant.

The distance of the objects that chiefly establish the mean values is approximately 1.3×10^8 light-years. From the region of minimum frequency to that of maximum frequency is therefore about 2×10^8 light-years—a transverse distance comparable to that over which Hubble found an apparent radial gradient. Since we find here the frequency increasing by

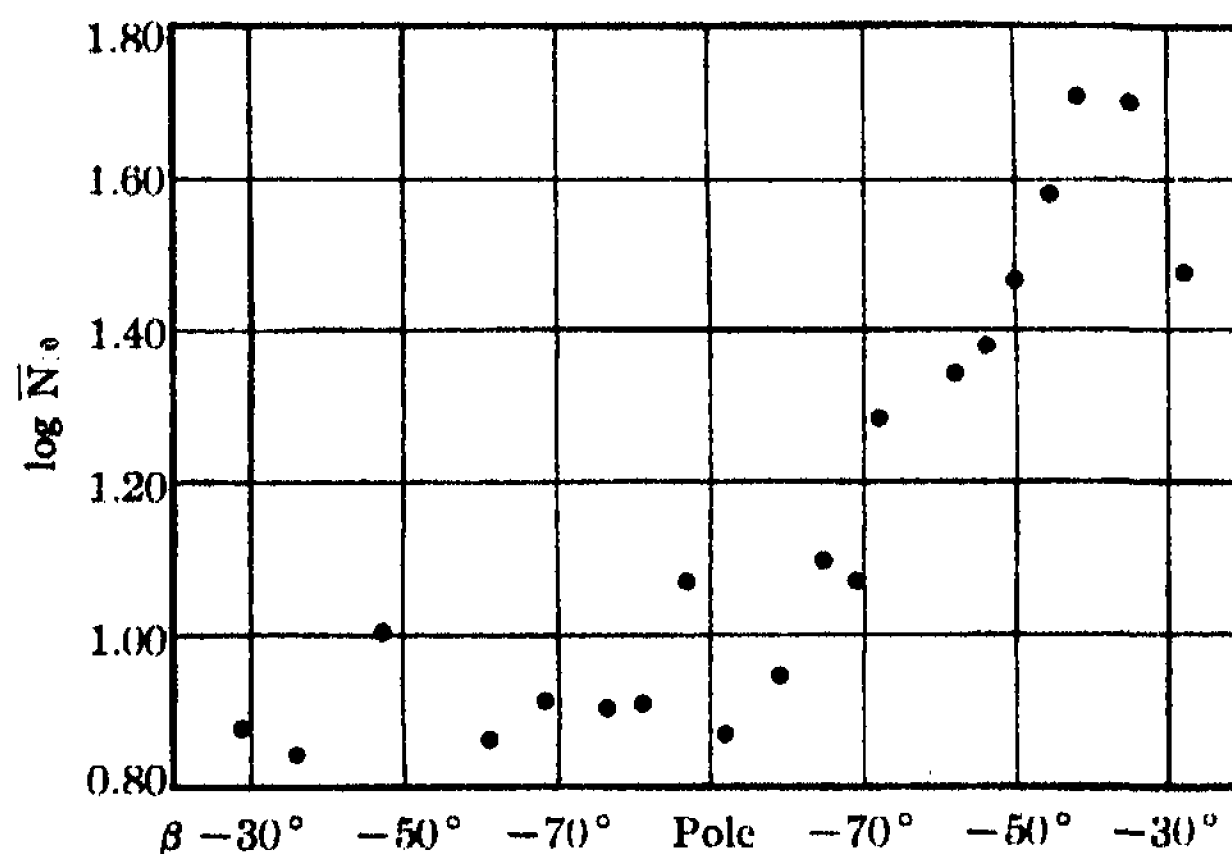


FIGURE 3

The metagalactic gradient as shown by a survey using the sixteen square degrees surrounding the central 9-square on a hundred plates. Galaxies only to a limit 0.3^m brighter than that for figure 2 are represented by this density curve.

several hundred per cent, it would appear reasonable to accept a twenty per cent radial density change as a minor detail of metagalactic structure, probably not associated significantly with the interpretation of the red-shift or with space curvature.

¹ *Harv. Bull.*, 894 (1934).

² *Harv. Ann.*, 105, Tercentenary Paper No. 8 (1937); see also *Harv. Ann.*, 88, No. 5 (1935).

³ *Mon. Not. R. A. S.*, 97, 508 (1937).

⁴ These PROCEEDINGS, 24, 148 (1938); *Harv. Circ.*, 423 (1937).

*A TARSIID PRIMATE AND A MIXODECTID FROM THE POWAY
EOCENE, CALIFORNIA*

BY CHESTER STOCK

BALCH GRADUATE SCHOOL OF THE GEOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF
TECHNOLOGY

Communicated June 7, 1938

Introduction.—Following the discovery and description of a titanotheri¹ in the Poway sands of San Diego County, continued excavation in this important Eocene horizon has produced small lots of mammalian remains. One of these included specimens of a tarsiid primate and a mixodectid. Worthy of mention is the fact that the former represents a third member of the Tarsiidae to be discovered in the early Tertiary of the Pacific Coast region. Since the Poway fauna possesses considerable significance in any attempt to correlate the later Eocene horizons of the Pacific Coast with those of the Cordilleran Province, it seems desirable to place on record these new mammals from the Poway.

***Yumanius woodringi*, n. gen. and n. sp.**

Type Specimen.—Right maxillary fragment with M_1 and M_2 , No. 2233, Calif. Inst. Tech. Vert. Pale. Coll., Plate 1, figure 1.

Paratypes.—Fragment of right ramus with M_1 – M_3 , No. 2234, Plate 1, figures 2, 2a and ramal fragment with M_2 , M_3 , No. 2235, Plate 1, figures 3, 3a.

Locality.—White sandstones associated with the Poway conglomerate and exposed on west bank of San Diego River, approximately one-quarter mile north and east of San Diego Mission; C. I. T. Vert. Pale., Loc. 249.

Generic and Specific Characters.—Upper molars differ from those of *Euryacodon lepidus* in possessing well developed and clearly defined hypocone or postero-internal cusp: Intermediate cusps also well developed. A second small cusp, comparable in size to that of the metaconule is situated between the latter and the base of the protocone. M_2 smaller than that in *Dyseolemur sylvestris* and hypocone, in contrast to that in the latter, more like a cingular cusp.

Lower molars 2 and 3 with trigonid portion of crown compressed antero-posteriorly and paraconid considerably reduced in size, more so than in *Euryacodon*. M_3 with broader posterior rim to heel. Distinctly different from *Dyseolemur* in reduction in size of trigonids in posterior molars and in absence of metastylid. I take pleasure in naming the species for my former colleague, Dr. Wendell P. Woodring of the U. S. Geological Survey.

Comparisons.—The upper molar teeth of *Yumanius woodringi* exhibit a cingulum on the outer, anterior and posterior sides of the crowns but the

basal ledge is lacking on the inner side. The small cuspule shown by Wortman² as situated at the inner base of the protocone in M_2 of *Euryacodon lepidus* is absent in the comparable tooth of *Y. woodringi*, but a slight development of the cingulum does occur in the second molar at the antero-internal base of the cusp. In the type specimen of *E. lepidus*, No. 11813 Y. P. M., the enamel of the crowns of the upper molars is smooth, the postero-internal cusp is not clearly indicated and the intermediate cuspules are small. In the molars of *Yumanius*, the postero-internal cusp shows pronounced development and the structural features of the crown become more complicated by the addition of a small cusp between metaconule and protocone. In addition the anterior ridge of the protocone, directed toward the protoconule, is more strongly formed in *Yumanius* than in *Euryacodon*.

The molars are unfortunately the only teeth preserved of the lower dentition. These resemble most closely the comparable teeth in *Euryacodon*. The enamel of the crowns is wrinkled, not smooth as in *Omomys*; the teeth are small, the paraconid while of normal size in M_1 becomes reduced to vestigial proportions or to the point of disappearance and the trigonid parts of the teeth are compressed anteroposteriorly. This compression of the trigonid portion of the crown is greater in the Poway genus than in *Euryacodon* or *Anaptomorphus*. In M_3 the posterior rim of the heel is broad and the end of the crown is not pointed as in *Euryacodon*. While no distinct metastylid is present as in *Dyseolemur* and *Washakius*, the posteriorly directed wing or ridge of the metaconid is extended in fore and aft line and is compressed transversely in *Yumanius*.

In the type of *Anaptomorphus aemulus* Cope, No. 5010 A. M. N. H., the teeth are smaller than in *Yumanius*. In M_1 of both forms the paraconid is well developed. This cusp becomes vestigial in M_2 , but its reduction and the anteroposterior compression of the trigonid is greater in *Yumanius*. In the lower molars of the Bridger genus the external cingulum is not so well developed as in the San Diego specimens.

Discussion.—The upper and lower jaw specimens with teeth herein referred to the new genus *Yumanius* were found at one locality in the Poway but were not directly associated. The view that the type and paratypes belong to two distinct tarsiid genera cannot be ignored, although apparent absence of evidence other than that of fortuitous occurrence makes this possibility a seemingly remote one. Presence of this material in the Poway gives strength to the belief that Wortman's reference of the three lower jaw fragments of a tarsiid from the Bridger to *Euryacodon lepidus* is correct. It follows, as Matthew³ pointed out, that *Anaptomorphus* and *Euryacodon* are probably identical. Among the several tarsiid genera recorded from the Bridger middle Eocene the Poway type is most closely related to the *Euryacodon-Anaptomorphus* stock. *Yumanius* appears to

have carried the line a stage farther and its presence at the San Diego locality suggests at least a post-Bridger age for the Poway. Curiously, *Y. woodringi* is more closely related to the Bridger species *E. lepidus* than to the Sespe type, *Dyseolemur pacificus*.

***Microsyops kratos*, n. sp.**

Type Specimen.—A left ramus with $Dp\bar{3}$, $P\bar{4}$ – $M\bar{3}$ inclusive and root fragments of the anterior premolars and canine, No. 2232, C. I. T. Vert. Pale. Coll., Plate 1, figures 4, 4a.

Locality.—Poway sandstones and conglomerates; C. I. T. Vert. Pale., Locality 249.

Specific Characters.—Differing from *Microsyops elegans* and *M. annectens* most noticeably in size. Smaller than *Craseops sylvestris*, but with lower molars, relative to size of jaw, larger than in latter species.

Description and Comparison.—This is the largest species of *Microsyops* so far recorded, being distinctly larger than *M. elegans* and *M. annectens* and resembling in size the species of *Craseops* recorded from the upper Eocene stage of the Sespe of Southern California. $Dp\bar{3}$ is a two-rooted tooth with simple crown. The latter is triangular in cross-section with apex placed forward. A downwardly directed ridge on the posterior surface of the principal cusp divides this surface into two parts. When the specimen was collected this tooth was loosely attached and on removal disclosed a small bit of enamel of a permanent tooth beneath. In front of $Dp\bar{3}$ is the exposed cross-section of the anterior premolar and presumably the canine. $P\bar{4}$ is the largest tooth of the series, $P\bar{4}$ – $M\bar{3}$ inclusive. In this tooth only a vestige of the paraconid ridge remains and the metaconid does not reach the height of the protoconid. The basin of the talonid is deeply excavated. This tooth and the molars which follow all show a well defined cingulum at the base of the protoconid and below the notch between this cusp and the hypoconid. In $M\bar{3}$ the posterior wall of the hypoconulid is broken away. The ramus is sturdy and of approximately same depth

PLATE 1

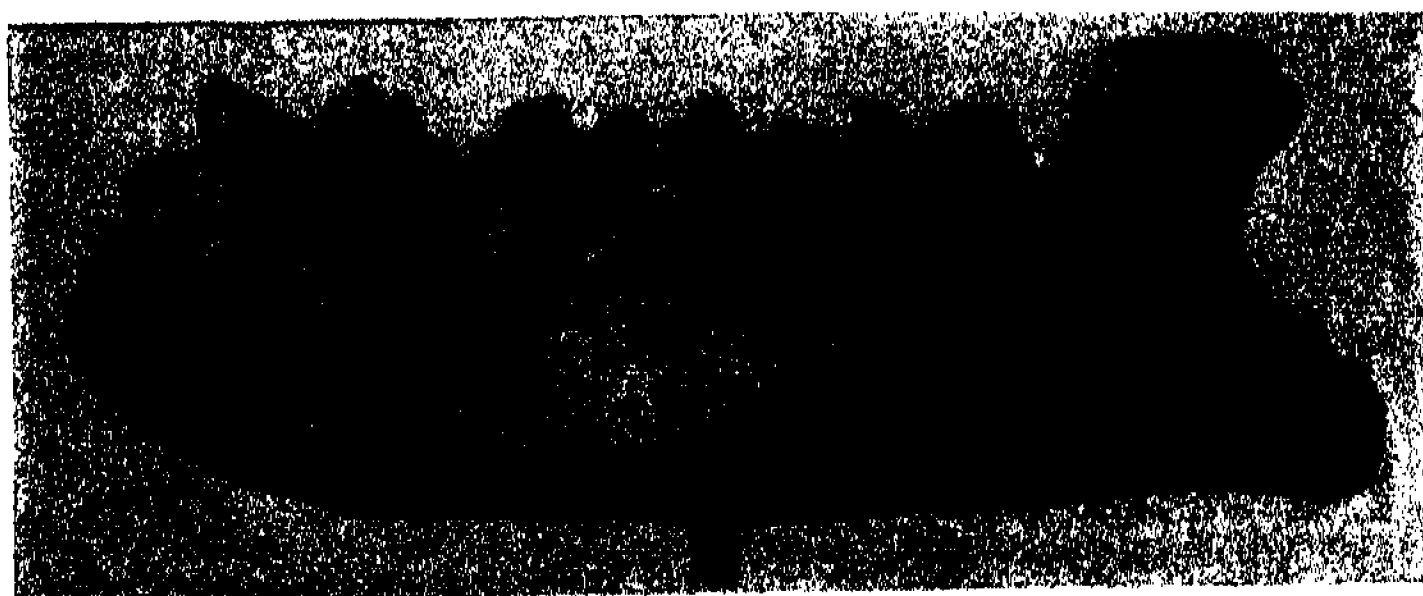
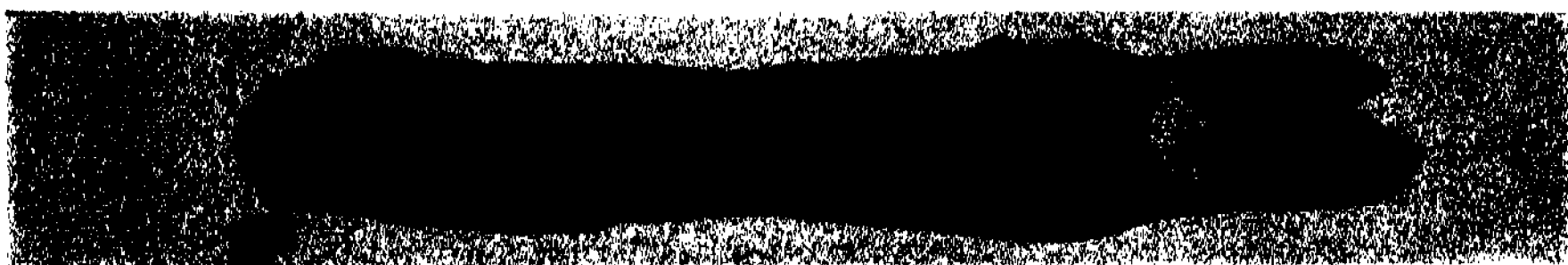
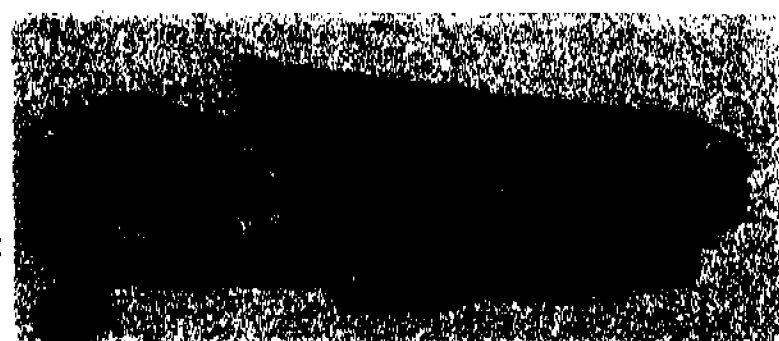
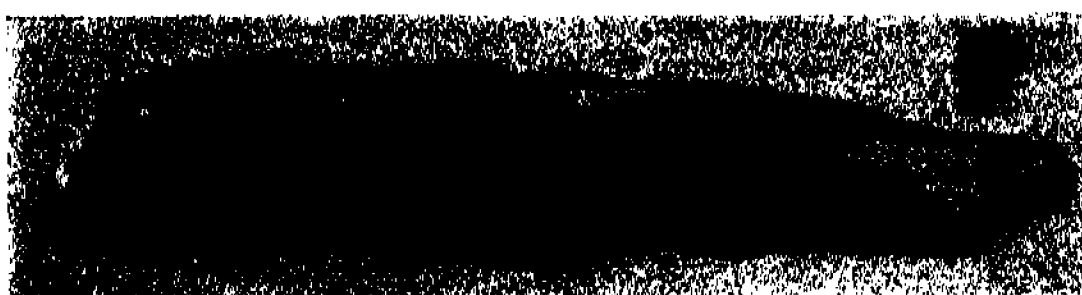
Yumanius woodringi, n. gen. and n. sp.

Figure 1, type specimen, No. 2233, skull fragment with $M\bar{2}$ and $M\bar{3}$, occlusal view. Figures 2, 2a, No. 2234, jaw fragment with $M\bar{1}$ – $M\bar{3}$, view of inner side and occlusal view. Note: In figure 2a, the paraconid of $M\bar{1}$ has been restored. Figure 3, 3a, No. 2235, outer and occlusal views. All figures approximately $\times 6$.

Microsyops kratos, n. sp.

Figures 4, 4a, type specimen, No. 2232, ramus with cheek teeth, lateral and occlusal views, $\times 2$.

Calif. Inst. Tech. Vert. Pale. Coll. Poway Eocene, San Diego Co., Calif.



throughout its length. Two mental foramina are situated below the anterior premolars.

Microsyops elegans from the Bridger, as represented by No. 12590, A. M. N. H., is a distinctly smaller, more slender form than the type from the Poway. In the former specimen the lower end of the symphyseal contact surface extends farther back than in No. 2232 from the Poway Eocene, and the second premolar is not crowded between adjacent teeth as in the latter. Moreover, there appears to be less discrepancy in length

COMPARATIVE MEASUREMENTS (IN MILLIMETERS)

	<i>Microsyops</i> <i>kratos</i>	<i>Microsyops</i> <i>elegans</i>	<i>Microsyops</i> <i>annectens</i>	<i>Craseops</i> <i>sylvestris</i>
	TYPE SPECIMEN No. 2232 C. I. T.	No. 12590 A. M. N. H.	TYPE SPECIMEN No. 11791 Y. P. M.	No. 1399 C. I. T.
Length from anterior end $Dp\bar{3}$ to posterior end of $M\bar{3}$	*28.7
Length from anterior end $P\bar{2}$ to posterior end of $M\bar{3}$	20.4
Length from anterior end $P\bar{3}$ to posterior end of $M\bar{3}$	18
Length from anterior end $P\bar{4}$ to posterior end of $M\bar{3}$	*24.8	15
$Dp\bar{3}$, length.....	3.9
$Dp\bar{3}$, width.....	2.4
$P\bar{4}$, length.....	6.6	3.6
$P\bar{4}$, width.....	4.2	2.6
$M\bar{1}$, length.....	5.5	3.5
$M\bar{1}$, width.....	4	2.4
$M\bar{2}$, length.....	6	6.3
$M\bar{2}$, width.....	4.2	3	4.6
$M\bar{3}$, length.....	* 6.2	4.5	5.7	7.1
$M\bar{3}$, width.....	3.9	3	3.5	4.5
Depth of ramus at posterior end of $M\bar{3}$	11.5	9	11.4	15
Depth of ramus at anterior end of $M\bar{2}$	10.9	8.2	14.2
Thickness of ramus below $M\bar{2}$	6.1	4.2	6.9
Thickness of ramus below $M\bar{3}$	6.2	4.5	8

* Approximate

between $P\bar{4}$ and $M\bar{1}$ in the Bridger species than in that from the San Diego locality.

No. 12050 A. M. N. H., also from the Bridger Eocene and determined as *Microsyops annectens*, approaches the Poway specimen in size. While the ramus exhibits a depth like that in the Californian type, its thickness is distinctly less.

No. 1399 C. I. T., representing *Craseops sylvestris* from the Sespe possesses a more massive ramus with the upper anterior border and the anterior end of the masseteric area decidedly more pronounced than in *Microsyops*

kratos. The two molar teeth preserved in No. 1399, $M\bar{2}$ and $M\bar{3}$, are slightly broader in comparison to their length in *Craseops* than in *Microsyops* from the Poway.

¹ Stock, C., *Proc. Nat. Acad. Sci.*, **23**, 48-53 (1937).

² Wortman, J. L., *Amer. Jour. Sci.*, Ser. 4, **17**, 139-140, fig. 133 (1904).

³ Matthew, W. D., and Granger, W., *Bull. Amer. Mus. Nat. Hist.*, **34**, 457 (1915).

LARGEST DEGREE OF A SUBSTITUTION IN THE GROUPS OF A GIVEN DEGREE

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated May 28, 1938

Let G represent a substitution group of degree n and let s represent a substitution of G which has the property that none of the substitutions of G has a degree which exceeds that of s . It follows directly from the average number of letters in the substitutions of a given group that the degree of s cannot be less than $n/2 + 1$. It has recently been proved¹ that when the degree of s has this smallest possible value, then all the substitutions of G besides the identity are of the same degree and G is an abelian group whose order is a power of 2. Moreover, n must then be of the form $2^m + 2^{m-1} + \dots + 2$ and there is one and only one such group for an arbitrary positive integral value of m . The order of this group is 2^m and it is conformal with the abelian group of type 1^m . Every substitution besides the identity of an arbitrary one of this infinite system of groups has exactly half of its letters in common with every other substitution besides the identity of this group whenever $m > 2$.

When the degree of s exceeds $n/2 + 1$ it is at least as large as $n/2 + 3/2$, and when it has this value there are only three possible groups, viz., the two groups of degree 3 and the nonabelian group of degree 5 and of order 6 whose transitive constituents are of degrees 2 and 3, respectively. As this fact was also established in the article to which we referred above, the smallest possible relative degree of s which has not been considered is $n/2 + 2$. When the degree of s is $n/2 + 1$ the group of degree 2 is the only possible transitive group, and when it is $n/2 + 3/2$ the two groups of degree 3 are the only possible transitive groups. It is obvious that when the degree of s is $n/2 + 2$ then the five transitive groups of degree 4 are the only possible transitive groups but there are then two intransitive groups in addition to three infinite systems of such groups whose degrees are of the

form $2^m + 2^{m-1} + \dots + 4$, $m > 2$, in two cases, and of the form $2^m + 1$ in the third case. In one of the first two cases the order of G is $2^m - 1$ while in the other it is 2^m . In the third case the order of G is also 2^m .

Each of the possible groups of degree 4 has the property that its substitutions of highest degrees are of degree $n/2 + 2$. Hence it may be assumed in what follows that $n > 4$ unless the contrary is stated. If at least one of the transitive constituents of G is of odd degree and the substitution of highest degree in G is of degree $n/2 + 2$, then two of the transitive constituents of G are of degree 3. Each of the remaining transitive constituents of G is then of degree 2 and hence the average number of letters in the substitutions of G is $n/2 + 1$. The value of n cannot exceed 8 because not more than four of the letters of the substitution of largest degree could appear in the group whose transitive constituents are 3, 3, and when $n = 8$ there are obviously two such groups of orders 6 and 18, respectively. Hence there are two and only two groups of degree n in which a substitution of highest degree is of degree $n/2 + 2$ and which involve separately at least one transitive constituent of odd degree. The transitive constituents of these groups are of degrees 2, 3, 3, respectively.

It remains to determine the groups of degree n whose substitutions of largest degrees involve $n/2 + 2$ letters and all of whose transitive constituents are of even degrees. The largest value of such a degree is 4 and not more than one of the transitive constituents of G can be of this degree. If G contains one such constituent and $n > 4$ its remaining constituents are of degree 2 and hence s involves at least $(n - 4)/2 + 1$ letters from this constituent. This is impossible since it involves 4 letters from the transitive constituent of degree 4. It results therefore that when the degree of s is $n/2 + 2$ and each of the transitive constituents of G is of even degree then all of these degrees are equal to 2 whenever $n > 4$. It remains only to consider the cases when each of the transitive constituents of G is of degree 2 and when G is therefore an abelian group.

The fundamental infinite system of such groups can then be constructed by choosing for s a substitution of degree 2^m , $m > 2$, and extending it by a substitution of the same degree which has half its letters in common with s just as in the case when the substitution of largest degree in G is of degree $n/2 + 1$. When $m > 2$ we extend this group of order 4 by a substitution which has half of its letters in common with each of the three substitutions of order 2 in this group of order 4. This process is repeated until each of the substitutions has four and only four letters in common with every other one while the separate pairs have half of their letters in common. The resulting group is of degree $2^m + 2^{m-1} + \dots + 4$ and of order $2^m - 1$. All of its substitutions, besides the identity, are of degree 2^m . By extending this group by a substitution of degree $2^m - 2$ which has two letters in common with each of its substitutions but involves no additional letter

there results a group of order 2^m and of degree $2^m + 2^{m-1} + \dots + 4$ whose substitution of largest degree involves $n/2 + 2$ letters.

Half of the substitutions of the latter group are negative and are of the same degree. The smallest two groups which belong to this system are the intransitive group of degree 4 and of order 4, and the group of degree 12 and of order 8 which involves four substitutions of degree 6 but no substitution of a larger degree than 8. To obtain the third infinite system of groups of degree n whose substitution of largest degree is of degree $n/2 + 2$ we adjoin to an arbitrary group of the former of the two infinite systems noted in the preceding paragraph a substitution of degree $2^m + 4$ which has two letters in common with each of its substitutions but involves four additional letters. The smallest group which belongs to this system is of order 4 and of degree 8. *There are therefore two infinite systems of groups of degree n in which the substitution of largest degree is of degree $n/2 + 2$ whenever n is of the form $2^m + 2^{m-1} + \dots + 4$. When n is of the form $2^m + 1$ there is an additional such system of groups.*

To prove that these three infinite systems of groups include all of the groups of degree n in which the substitutions of largest degree are of degree $n/2 + 2$ and each of the systems of intransitivity is of degree 2, it is desirable to consider separately the three cases in which G is composed of the substitutions of highest degree together with the identity, in which the substitutions of highest degree generate a proper subgroup of G , and in which these substitutions generate G but G involves also substitutions which are not of highest degree. In each of these cases G involves at least $n/4$ substitutions of highest degree since the average number of the letters in its substitutions is $n/2$ and hence it must involve $n/4$ substitutions of highest degree to account for the fact that the identity is of zero degree, since each substitution of highest degree involves two more letters than the average.

When all the substitutions of G besides the identity are of degree $n/2 + 2$ then this degree is 2^m since every two substitutions of order 2 contained in G have half of their letters in common. It therefore results that $n = 2^m + 2^{m-1} + \dots + 4$ and the order of G is $2^m - 1$. Hence G is one of the groups of the first of the three infinite systems noted above whenever it is composed of its substitutions of highest degree together with the identity. It is obvious that a substitution s which has two letters in common with each of the substitutions of order 2 in such a G is negative and of degree $n/2$. The products of s into all the substitutions of G give all of the substitutions which have this property with respect to a given G and hence there results a group which is completely determined by the given G and of twice its order. This group belongs to the second of the infinite system noted above and all of the groups which can be formed in this way constitute this system.

It remains to consider the case when the substitutions of highest degree

in G generate G but generate also substitutions of lower degree. In this case the substitutions of highest degree are negative since this degree is a power of 2 increased by 2. In fact, if the degree of these substitutions is a power of 2 increased by $2k$ and we construct G as noted above the substitution of highest degree in the resulting group is of the form $n/2 + k + 1$. In this construction we extend successively the group formed by the substitution built on the part of a substitution of largest degree whose degree is a power of 2. It therefore results that the three infinite systems of groups noted above include all the groups of degree n which involve only systems of intransitivity of degree 2 and whose substitutions of largest degree involve exactly $n/2 + 2$ letters. The orders of the groups of the last system are one-half of their degrees.

When the largest degree of a substitution in a group of degree n exceeds $n/2 + 2$ it is at least as large as $n/2 + 5/2$ and when it has this value n is odd and the number of possible groups is infinite since if there is a transitive constituent of degree 3 there is an infinite number of groups on the remaining $n - 3$ letters whose substitutions of largest degree are $(n - 3)/2 + 1$. Hence there is an infinite number of groups on n letters whose substitutions of largest degree are of degree $(n - 3)/2 + 4$ or $n/2 + 5/2$. Such groups can be constructed by forming the direct products or by establishing an isomorphism between the symmetric group of degree 3 and a group whose order is a power of 2 and whose substitutions of largest degree are of degree $(n - 3)/2 + 1$. They can also be constructed by forming the direct products of the alternating group of degree 3 and the latter groups as well as by the direct products of the three groups of degree n whose substitution of largest degree involves $n/2 + 3/2$ letters.

It has been noted that the smallest relative number of letters in a group of degree n appears only when n is of the special form $2^m + 2^{m-1} + \dots + 2$, and that when n is of the form $2^m + 1$ then there are groups whose substitutions of largest degree do not involve more than $n/2 + 2$ letters. From the latter of these theorems it results directly that for an arbitrary value of n there is a substitution group of degree n which does not involve more than $3n/4 + 2$ letters since there is a number which is a power of 2 and exceeds $n/2$. This degree is usually much smaller as follows from repeated applications of the given results.

¹ *Proc. Nat. Acad. Sci.*, 24, 202-204 (1938).

REMARKS ON RIEMANN'S DOCTORAL DISSERTATION

BY JESSE DOUGLAS

BROOKLYN, N. Y.

Communicated June 10, 1938

1. The author has based the solution of Plateau's problem on the minimum principle

$$\begin{aligned} D(H) &= \int \int \frac{1}{2} (E + G) du dv \\ &= \frac{1}{2} \int \int \sum_{i=1}^n \left[\left(\frac{\partial H_i}{\partial u} \right)^2 + \left(\frac{\partial H_i}{\partial v} \right)^2 \right] du dv = \min., \end{aligned} \quad (1)$$

among all harmonic vectors $H(u, v)$ which represent a surface bounded by a given contour Γ in n -dimensional euclidean space.¹ The (u, v) domain may be supposed to be the unit circle C .

Explicitly, $D(H)$ may be expressed in terms of the boundary values $g(\theta)$ of H on the unit circumference c by the formula

$$A(g) = \frac{1}{4\pi} \int_c \int_c \frac{\sum_{i=1}^n [g_i(\theta) - g_i(\varphi)]^2}{4 \sin^2 \frac{\theta - \varphi}{2}} d\theta d\varphi. \quad (2)$$

Since, for given boundary values, a harmonic function gives the least value to Dirichlet's integral, the principle (1) is equivalent to

$$\begin{aligned} D(\mathfrak{r}) &= \frac{1}{2} \int \int (\mathfrak{r}_u^2 + \mathfrak{r}_v^2) du dv = \\ &= \frac{1}{2} \int \int \sum_{i=1}^n \left[\left(\frac{\partial x_i}{\partial u} \right)^2 + \left(\frac{\partial x_i}{\partial v} \right)^2 \right] du dv = \min., \end{aligned} \quad (3)$$

where the vector $\mathfrak{r}(u, v)$ may represent any piece-wise continuously differentiable surface bounded by Γ .

If $n = 2$, so that Γ is a plane curve enclosing a region R , then, as was observed by the author, the Plateau problem becomes the one of mapping R on C conformally. Denote by $x(u, v)$, $y(u, v)$ the components of \mathfrak{r} ; then

$$\mathfrak{A} = \int \int_C (x_u y_v - x_v y_u) du dv = \frac{1}{2} \int_{\Gamma} (x dy - y dx) \quad (4)$$

is a constant, equal to the area of R , for all vectors $x(u, v)$, $y(u, v)$ in the unit circle which map the circumference c topologically on Γ .² Hence the principle (3) is equivalent to

$$D(\mathfrak{x}) - \mathfrak{A} = \min., \quad (5)$$

or

$$E(x, y) = \int \int_C [(x_u - y_v)^2 + (x_v + y_u)^2] du dv = \min. \quad (6)$$

Indeed, for a conformal map the Cauchy-Riemann equations

$$x_u - y_v = 0, \quad x_v + y_u = 0, \quad (7)$$

are satisfied, and $E(x, y) = 0$; while for a non-conformal map, $E(x, y) > 0$.

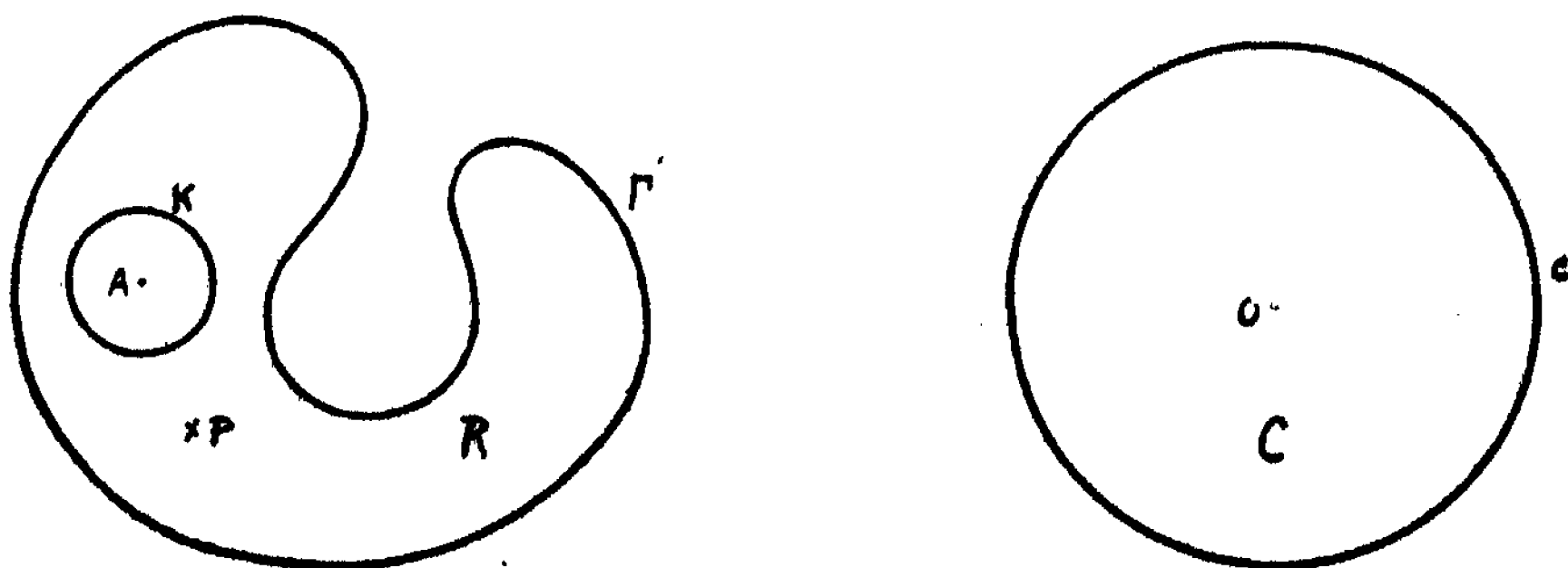
The resemblance which the minimum principle (6) bears to the one considered by Riemann in his dissertation:³

$$\Omega(\omega) = \int \int_R [(\omega_x - \beta_v)^2 + (\omega_v + \beta_x)^2] dx dy = \min., \quad (8)$$

is only superficial. But recently the statement has been made, in reference to (6), that: "This is exactly the famous variational problem which Riemann considered in his doctor's thesis."⁴

In view of the classic character of Riemann's dissertation, and its influence on the entire subsequent development of the theory of conformal mapping, it therefore seems appropriate to point out a number of sharp and essential differences between the principles (6) and (8). To do this is the purpose of the present note.

2. Riemann was seeking a minimum principle to use as a basis for establishing the existence of Green's function $G(P, A)$ of the region R , or the equivalent conformal map of R on the circular region C .



The Green's function is uniquely defined by the properties: (1) harmonic in R ; (2) vanishing on Γ ; (3) regular in the interior of R , except for a logarithmic singularity at the point A :

$$G(P, A) = \log \rho + h(P, A), \quad (9)$$

where ρ = distance AP , and h is a harmonic function of the point P regular throughout R including the point A . If G_1 denote the conjugate harmonic function to G , i.e.,

$$G_1(P) = - \int_{P_0}^P \frac{\partial G}{\partial n} ds, \quad (10)$$

then the conformal transformation from R to C is given by the function $e^{G + iG_1}$.

If Γ is a general type of boundary curve, then the behavior of $\partial G / \partial n$, therefore of G_1 , as we approach the boundary is uncertain. Hence the conformal map furnished by Riemann's method subsists only between the *interiors* of the regions involved, and the problem of what type of correspondence is established between the *boundaries* is left open. This problem was solved many years after the work of Riemann by Osgood and by Carathéodory,⁵ who proved that the conformal map of the interiors induces by continuity a one-one continuous correspondence between the boundaries.

3. The first suggestion for determining G by means of a minimum condition might be to use the Dirichlet principle as it stands:

$$D(\omega) = \iint_R (\omega_x^2 + \omega_y^2) dx dy = \min., \quad (11)$$

where we admit to comparison all functions $\omega(x, y)$ of like nature to $G(P, A)$: vanishing on the boundary Γ , and logarithmically singular at A . But since, as is easily verified,

$$D(\log \rho) = +\infty \quad (12)$$

over any neighborhood of A , we have the fundamental difficulty that

$$D(\omega) \equiv +\infty \quad (13)$$

for all functions ω logarithmically singular at A . Hence the original problem (11) *has no sense*.

We need a functional that for at least one value of its argument takes a finite value. Riemann secures this by means of an ingenious device, in the use of which (or similar devices) he has been followed by many subsequent contributors to the theory.⁶

He introduces, instead of $D(\omega) = \min.$, the problem (8) $\Omega(\omega) = \min.$, where β is a *fixed* function so designed that $\Omega(\omega)$ is finite for at least a particular form of ω . *Regardless of the parameter function β* , this problem has the same variational equation as $D(\omega) = \min.$, namely,

$$\frac{\partial}{\partial x} (\omega_x - \beta_y) + \frac{\partial}{\partial y} (\omega_y + \beta_x) = \omega_{xx} + \omega_{yy} = 0, \quad (14)$$

or Laplace's equation. The function β simply cancels out in the formation of the variational condition.

Riemann's idea was to arrange β so that $\Omega(\omega)$ will be finite for at least one particular form of ω . This he accomplished by choosing β to coincide in a small circle K about A as center with the conjugate harmonic function to the one, $\log \rho$, that characterizes the singularity at A , namely,

$$\beta(x, y) = \tan^{-1} \frac{y}{x} \quad (15)$$

(we have taken A to be the origin). In $R - K$, β may be any smooth function which (1) like $\tan^{-1} y/x$, has a period of 2π with respect to a cut L in R that leads from A to the boundary Γ , and (2) which attaches smoothly to $\tan^{-1} y/x$ at the circumference of K . The simplest choice is to define β by the formula (15) throughout the region R as provided with the cut L . That β is multiform is no serious inconvenience, since only its partial derivatives are used, which are uniform:

$$\beta_x = \frac{-y}{x^2 + y^2}, \quad \beta_y = \frac{x}{x^2 + y^2}. \quad (16)$$

Riemann then selects as particular form of ω a function α defined and smooth throughout R except at the point A , equal to $\log \rho$ in the circle K , and reducing to zero on Γ .⁸ Then, precisely because of the prearranged conjugacy between β and $\log \rho$ in the circle K , we have in that circle.

$$\alpha_x - \beta_y = 0, \quad \alpha_y + \beta_x = 0; \quad (17)$$

therefore

$$\Omega(\alpha) = 0 \text{ over } K. \quad (18)$$

Since the partial derivatives of α and β are bounded and continuous⁹ in the rest of the region, $R - K$, it follows that

$$\Omega(\alpha) = \text{finite quantity}. \quad (19)$$

Thus Riemann concludes that the problem (8) *has sense*. The minimizing function ω^* , if existent,¹⁰ will then obey Laplace's equation, and this, together with its vanishing on Γ and logarithmic singularity at A , characterizes ω^* as Green's function $G(P, A)$.

4. The preceding discussion may be summarized as follows.

In the problem $\Omega(\omega) = \min.$ considered by Riemann, β is *entirely fixed*, and the *only* function in competition is ω , which varies subject to the *fixed boundary values zero* and logarithmic singularity at A .

But in the problem $E(x, y) = \min.$,

(1) $x(u, v)$, $y(u, v)$ are *both variable* in competition with other function-pairs;

(2) the boundary values of x, y are *not fixed*, but vary simultaneously subject to the restriction of always defining a topological correspondence between Γ and c .

More important is the following distinction.

(3) In Riemann's problem, the argument function $\omega(x, y)$ is defined in the *generally complicated region* R , and the sole and entire purpose of the problem is to obtain the Green's function of R by identifying it with the minimizing function ω^* .

But in the problem $E(x, y) = \min.$, the argument functions are defined in the *circular region* C , where we have no need to find Green's function, since that is known by elementary geometry:

$$G(P, A) = \log \frac{AP}{A_0P} - \log \frac{AI}{A_0I}, \quad (20)$$

A_0 denoting the inverse point of A , and I the point $(1, 0)$.

Since $E(x, y)$ differs only by a constant from the sum of Dirichlet integrals $D(x) + D(y)$, the minimizing functions $x(u, v)$, $y(u, v)$ must be harmonic, and are therefore determined by Poisson's integral as soon as their *boundary values* $x(\theta)$, $y(\theta)$ are known. Thus, the real point in connection with $E(x, y) = \min.$ is to find what topological correspondence shall be established by $x(\theta)$, $y(\theta)$ between Γ and c .

The author has expressed this in an explicit way by the principle (2) $A(g) = \min.$, where g denotes the vector with the components $x(\theta)$, $y(\theta)$. The *direct* treatment of this minimum problem is safe from the Weierstrass criticism,¹¹ since the argument range $[g]$ is *compact*,¹² whereas the argument range $[\omega]$ of Riemann's problem is *not*.¹³ Accordingly, the existence of the minimizing vector $x^*(\theta)$, $y^*(\theta)$ is easily established, and the conformal transformation from C to R is then given immediately by the Poisson integrals for $x^*(\theta)$ and $y^*(\theta)$, or by the equivalent Cauchy formula

$$W = \frac{1}{2\pi i} \int_c [x^*(\theta) + iy^*(\theta)] \frac{dz}{z - w} \quad (z = e^{i\theta}). \quad (21)$$

(4) The minimum problem of Riemann gives, when solved, only a correspondence between the *interiors* of R and C . Supplementary considerations of quite different character were necessary to extend this correspondence by continuity to the *boundaries*. In fact, the topological nature of the boundary correspondence was only established by Osgood and by Carathéodory a number of years after the rigorous solution of the Riemann-Dirichlet minimum problem by Hilbert.¹⁴

But in the problem $E(x, y) = \min.$, the very nature of the argument functions, as prescribed, must *automatically* give a topological correspon-

dence between the boundaries simultaneously with the conformal map of the interiors.

5. In conclusion, no basis remains for identifying the problem (6) $E(x, y) = \min.$ with the one considered by Riemann in his famous dissertation.

¹ J. Douglas, *Trans. Amer. Math. Soc.*, **33**, 263-321 (1931); *Bull. Amer. Math. Soc.*, **39**, 227-251 (1933).

² This is regardless of whether the locus of the point (x, y) covers the region R simply or multiply, or extends outside that region. To assure the existence of the curvilinear integral in (4), we may suppose Γ to be rectifiable, but the fact of the constancy of α applies when Γ is any Jordan curve.

³ B. Riemann, *Grundlagen für eine allgemeine Theorie der Functionen einer veränderlichen complexen Grösse*, Göttingen, 1851. *Gesammelte Werke*, 1892. See especially §§16, 18, 21.

⁴ R. Courant, *Ann. Math.*, **38**, 696 (1937), where the transformation from (3) to (6) is given. See also Courant-Hilbert, *Methoden der Mathematischen Physik*, **2**, 536-537 (1937).

⁵ W. F. Osgood and E. H. Taylor, *Trans. Amer. Math. Soc.*, **14**, 277-298 (1913); C. Carathéodory, *Math. Annalen*, **73**, 305-320 (1913).

⁶ Notably Weyl; see *Die Idee der Riemannschen Fläche*, 1913, p. 92.

⁷ One whose radius is less than the distance from A to Γ .

⁸ In Riemann's presentation, $\alpha + i\beta$ is chosen equal to $\log(x + iy)$ in the circle K (called Θ by Riemann), which is equivalent to the separate conditions $\alpha = \log \rho$, $\beta = \tan^{-1} y/x$. Riemann's stipulation (loc cit., p. 41) that $\alpha + i\beta$ be pure imaginary on the boundary of R is, of course, equivalent to: $\alpha = 0$ on Γ . The region R is called T by Riemann.

⁹ We may even take α to be zero in a region adjacent to the boundary Γ , as well as upon it.

¹⁰ We have in mind the remark of Weierstrass, which emphasized the necessity of proving rigorously the attainment of the minimum. This was not accomplished until about 1900 by Hilbert, "Über das Dirichletsche Prinzip," *Math. Annalen*, **59**, 161-186.

¹¹ See footnote 10.

¹² Every sequence of elements g contains a convergent sub-sequence.

¹³ For a fuller discussion of this point, see the second reference in footnote 1.

¹⁴ See footnotes 5 and 10.

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

August 15, 1938

Number 8

Copyright 1938 by The National Academy of Sciences

THE ORIGIN OF MAIZE

BY P. C. MANGELSDORF AND R. G. REEVES

TEXAS AGRICULTURAL EXPERIMENT STATION AND A. & M. COLLEGE OF TEXAS, COLLEGE
STATION, TEXAS

Communicated July 5, 1938

To botanists and archaeologists alike, the origin of Indian corn or maize has been a most intriguing problem, for maize was the basic food plant of the ancient American civilizations. Both DeCandolle¹ and Darwin² concluded that maize had its origin in America and the possibility of a pre-Columbian distribution in China has since been dismissed by Laufer's comprehensive studies.³

The wild form of maize is unknown and perhaps is now extinct. No fossil remains are known, the one supposedly fossil ear⁴ having recently been shown to be an artifact.⁵ In the absence of evidence from these and other sources there is left only one recourse in an attack on the problem, a study of the plant itself and of its relatives. Since evolution is primarily a matter of gene change and chromatin rearrangement, we have assumed that cytogenetic investigations of the hybrids of maize and its relatives would provide the most promising avenue of attack upon this problem.

The tribe Maydeae to which maize is assigned comprises eight genera, of which only three, *Zea*, *Euchlaena* and *Tripsacum*, are American. The relationship of the Oriental genera to maize is remote, and they appear to have no immediate bearing on the problem of the origin of maize. *Euchlaena*, *E. mexicana* Schrad., or teosinte, is an annual confined to Mexico and western Guatemala. In Mexico, it occurs as a weed in and around the corn fields, but in Guatemala, in at least one locality, it is found as a truly wild species.⁶ A perennial, tetraploid form, *E. perennis* Hitchc., is also known but it occurs only in a single colony in Mexico and is of questionable specific rank. *Tripsacum*, a perennial comprising six or seven species, is widely distributed from Central America through the southern and eastern states, and is occasionally found in parts of South America.

Three general theories with regard to the origin of maize have had a vogue: (1) That it originated from pod-corn, which differs from normal maize by a single dominant gene governing the development of a brittle,

readily disarticulating rachis and the production of prominent glumes enclosing the seeds (cf. Saint-Hilaire,⁷ Sturtevant⁸). (2) That maize originated from teosinte by direct selection, by large scale mutations or by the hybridization of *Euchlaena* with some unknown grass (cf. Harshberger,⁹ Collins¹⁰). (3) That maize, teosinte and *Tripsacum* have descended along independent lines from a remote common ancestor (cf. Weatherwax¹¹).

The rather general acceptance of the theory that *Euchlaena* has played some rôle in the ancestry of maize has led to the assumptions that maize had its origin in Central America or Mexico, and that agriculture, which preceded all advanced cultures and civilizations in the New World, also had its origin there (cf. Spinden¹²).

Our cytogenetic studies covering an eight-year period have led to the surprising conclusion that *Euchlaena*, far from being the progenitor of maize, is the descendant of a natural hybrid of *Zea* and *Tripsacum*.

Our experiments began with hybrids of *Zea* and *Euchlaena* made to determine how the genes which differentiate the two genera are distributed on the chromosomes. Most orthodox geneticists assume that differences between genera and species are genic. Hybrids of *Zea* and *Euchlaena* furnish excellent material, not only for testing this assumption but also for determining how the genes which differentiate the genera are distributed on the chromosomes. *Zea* and *Euchlaena*, differing widely in many quantitative characteristics, have the same chromosome number, 10, hybridize readily, producing highly fertile hybrids, where chromosome pairing is almost complete, and where crossing-over occurs almost as readily as in pure *Zea*.¹³ Finally, there are available for each of the ten chromosomes of maize two or more well-known genes which may be used as markers.

The procedure was to cross *Euchlaena* with stocks of maize in which certain chromosomes were each marked by two genes, and then to back-cross the F_1 hybrid to a double recessive maize stock. In the succeeding generation of each test four classes of plants appeared, two of which were cross-over classes, the other two parental combinations. Plants which exhibited both of the marker genes from *Zea* had presumably inherited intact, except for rare double cross-overs, the section of chromatin lying between these two genes, while plants which exhibited the two alleles from *Euchlaena* had received intact a corresponding section from *Euchlaena*. As these two groups of plants were, on the average, alike in all other genes and in their cytoplasm, a comparison of the two groups in various quantitative characteristics by which the two parental genera differ, is essentially a comparison of two corresponding regions of the chromatin in the two genera.

Tests of this nature have been completed with only four of the ten chromosomes; but these show (1) that the differences between *Zea* and *Euchlaena* are genic; (2) that the genes which differentiate the two genera are not distributed at random on all chromosomes. The genes on chromosome No. 2

marked by *B* and *lg*, 34 units apart, on chromosome No. 6 marked by *Y* and *Pl*, 28 units apart and on chromosome No. 9 marked by *wx* which occupies a region approximately 45 units in length which does not cross-over regularly with the *Euchlaena* homolog,¹⁴ have but little effect upon the quantitative characteristics which differentiate *Zea* and *Euchlaena*. But the genes on chromosome No. 4 marked by *su* and *Tu*, 29 units apart, have a decided effect upon almost all the quantitative characteristics by which the two genera were distinguished in this study.

This non-randomness becomes explicable only after the results of hybridizing *Zea* and *Tripsacum* have been considered. Hybridization of these two genera is rather difficult but can be accomplished by the use of a simple technique which involves shortening the styles or silks of maize.¹⁵ With this procedure hybrid seeds are produced in abundance, and though most of these abort at an early stage, a few reach maturity and are capable of germination.

The F_1 hybrids are quite vigorous but completely pollen-sterile. Studies of meiosis show very little pairing between the 10 *Zea* and the 18 *Tripsacum* chromosomes although a feeble association is occasionally observed. Unreduced gametes are regularly formed and are apparently the only ones which function. The hybrid sets a small percentage of seeds when pollinated with either of the parental genera or with the third genus, *Euchlaena*. As the backcross to *Zea* is the only one which has a direct bearing on the origin of maize, it is the one which will be considered here.

This triploid hybrid possesses 38 chromosomes of which 20 are derived from *Zea*; 18 from *Tripsacum*. At meiosis the 20 *Zea* chromosomes pair completely to form 10 bivalents which behave quite regularly. The 18 *Tripsacum* chromosomes remain unpaired except that there is an occasional feeble association of one or several *Tripsacum* chromosomes with the *Zea* bivalents. The triploid hybrid is completely pollen-sterile but exhibits a fertility of 21 per cent when pollinated by *Zea*.

In the succeeding generation all plants possess 20 *Zea* chromosomes plus (usually) one or more *Tripsacum* chromosomes. The distribution of 178 plants in this population, with respect to the number of *Tripsacum* chromosomes is as follows: 0;25, 1;76, 2;22, 3;4, 4;8, 5;3, 6;3, 7;4, 8;6, 9;5, 10 or more; 22. Plants with no extra *Tripsacum* chromosomes are usually typical maize plants with no evidence of *Tripsacum* influence. A few $2n$ plants, however, exhibit a slight trace of *Tripsacum* influence, indicating the presence of a few *Tripsacum* genes. As the number of *Tripsacum* chromosomes increases, the *Tripsacum* influence becomes more perceptible; but the relationship is not linear, since one or two *Tripsacum* chromosomes have a much greater relative effect than a larger number.

It had been planned to isolate from this population 18 different $2n + 1$ stocks in which each of the 18 *Tripsacum* chromosomes was represented

once, so that the effects of each of the *Tripsacum* chromosomes could be studied separately, but as the *Tripsacum* chromosomes were never transmitted through the pollen, and as there was a marked selective action against their transmission through the ovules, many of the stocks were lost. Rather comprehensive studies were completed, however, in one of the $2n + 1$ stocks in which the *Tripsacum* chromosome was marked by an allele of the *su* gene from maize chromosome No. 4. Prophase studies of the $2n + 1$ plants segregating from this stock showed that the *Tripsacum* chromosome paired most frequently with *Zea* chromosome No. 1 but it was also observed pairing with Nos. 4, 5 and 10.

In a stock that is homozygous for the maize gene *su*, the *Tripsacum* chromosome bearing the allele of *su* is identified by starchiness of the seeds. Starchy seeds when planted give rise to $2n + 1$ plants; sugary seeds from the same ears produce normal $2n$ maize plants. Exceptions to this rule have shown that there is sometimes an exchange of chromatin between the *Tripsacum* chromosome and one or more of the maize chromosomes. We have had $2n$ plants which exhibited a definite *Tripsacum* influence including the allele of *su*, and $2n + 1$ plants with irregularities in addition to those attributable to the extra chromosome.

The evidence that there can be an interchange of *Zea* and *Tripsacum* chromatin has led to a consideration of the possible consequences of a natural hybridization of *Zea* and *Tripsacum*. Obviously there would have been a rapid elimination of the unbalanced types and a quick return to the two parental types; pure *Tripsacum*, or *Tripsacum* with a few *Zea* genes; pure *Zea* or *Zea* with a few *Tripsacum* genes. The infected *Tripsacum* might have been slightly less able to survive in nature than pure *Tripsacum*; the infected *Zea* would have been better fitted to survive in nature only if the *Tripsacum* infection had provided certain characteristics which maize now lacks, particularly a means of dispersal and protection for the seed, characteristics which *Tripsacum* possesses in its prominent, horny glumes and brittle rachis.

A description of a maize plant possessing these two *Tripsacum* characteristics is obviously a rough description of a plant already in existence, *Euchlaena*; consequently we began to consider seriously for the first time an unpublished suggestion made by Dr. Edgar Anderson, that *Euchlaena* might be the product of the natural hybridization of *Zea* and *Tripsacum*.

A consideration of this hypothesis reveals an amazing amount of evidence in its support, of which the most obvious is the fact that *Euchlaena* is intermediate between, or identical with, its two putative parents in all of its characteristics. It does not possess, apparently, a single characteristic which might not have been received from either *Tripsacum* or *Zea*. If *Euchlaena* represents the end-product of an independent line of descent from a remote common ancestor, it is almost inconceivable that it should

not have acquired some distinctive characteristics in which it differed from both *Zea* and *Tripsacum*.

Convincing evidence of the hybrid nature of *Euchlaena* has come from a reëxamination of the segregates from hybrids of *Zea* and *Euchlaena* which has revealed that *Euchlaena* differs genetically from *Zea* primarily by four segments of chromatin which bear genes with *Tripsacum* effects. The material involved in these studies is the same which had previously been used to study linkage between quantitative and qualitative characters, as reported earlier in this paper. The previous studies had been made on the assumption that *Zea* and *Euchlaena* are distinct genera and that the numerous quantitative differences between them are generic differences. Consequently we had measured as many as possible of the characteristics in which the two genera differed. In reapproaching the problem we attempted to ignore all differences of the kind which occur within *Zea* itself and to confine ourselves to the essential characteristics which distinguish *Euchlaena* from *Zea*. These are only four: prominent horny glumes, a brittle rachis, unpaired pistillate spikelets and a distichous arrangement of the spikelets of the pistillate inflorescence. As *Zea* is dominant for paired spikelets and the F_1 hybrid had been back-crossed to *Zea*, it was impossible to study the segregation for this character in this material. In attempting to arrive at a classification with respect to the remaining characteristics, numerous difficulties were encountered, but it finally became apparent that segregates possessing none of these characteristics and hence essentially typical maize plants were occurring in a ratio of approximately 1 in 16 while segregates possessing all of these characteristics and duplicating the F_1 hybrids were occurring in approximately the same ratio. This frequent reappearance of the parental types indicated that only a relatively small number of factors could be involved and the 15:1 ratio suggested a four-factor-segregation which in a back-cross should result in five distinct classes occurring in a ratio of 1:4:6:4:1. It was found that the segregates could be readily classified according to such a scheme, the actual numbers of plants falling into the different classes being 22, 190, 384, 303 and 21. Although the distribution differs significantly from the theoretical ratio, there is little doubt that the classification is essentially correct, for the deviations are largely accounted for by several disturbing influences. Furthermore, each class can be divided into its component sub-classes, and it has been possible to identify the 16 distinct classes expected from a four-factor segregation.

Although the population segregated in a four-factor ratio, it is scarcely conceivable that the differences between *Zea* and *Euchlaena* are due alone to four genes. Certain exceptional plants which exhibit part, but not all, of the effects of one of these factors indicate that each factor represents a group of genes, probably a short section of chromatin usually inherited

intact, which bears *Tripsacum* genes, or genes with *Tripsacum* effects. We assume that these segments of chromatin have been received originally from *Tripsacum* as the result of translocations between *Zea* and *Tripsacum* chromosomes.

Since these translocation segments are usually inherited intact, they may be treated as single genes. Linkage studies with the genes which had previously been used to mark the four chromosomes included in these studies show that none of the segments occur on chromosomes 2, 6 or 9, but that one, possibly two, of them are located on chromosome 4. Translocation segment A shows 29 per cent of crossing-over with *Tu* and 34 per cent with *su*. Segment C shows 38 per cent of crossing-over with *su* and 47 per cent with *Tu*. T-A and T-C exhibit 53 per cent of crossing-over with each other and *su* and *Tu* show 28 per cent.

Though the classification of this population into 16 distinct classes was achieved only by concentrating on the essential differences between *Zea* and *Euchlaena* and ignoring all others, yet once the classification was made, it was found that all other differences previously measured were associated with these four translocation segments. There is, in most cases, a linear relationship between the number of translocation segments and the quantitative characteristics which differentiate the two genera. In several characteristics the translocation segments account for almost all of the differences between *Zea* and *Euchlaena*. We may conclude, therefore, that *Euchlaena* differs from *Zea* primarily by four segments of chromatin, which, because they bear genes with *Tripsacum* effects, are assumed to have been received originally from *Tripsacum* as the result of natural hybridization of *Zea* and *Tripsacum* followed by back-crossing to *Zea*. Two of these segments, which account for approximately half of the differences between *Zea* and *Euchlaena*, appear to be located at opposite ends of Chromosome No. 4 and this accounts for the fact, previously noted, that the genes which differentiate *Zea* and *Euchlaena* are not distributed at random but are concentrated on chromosome No. 4.

Though the archaeological problems of Central America are far from final solution, there are several well-established facts, which, considered with the botanical evidence, suggest that the hybridization of *Zea* and *Tripsacum* which give rise to the new genus *Euchlaena* probably occurred after the year 600 A. D. *Euchlaena* is unknown and *Tripsacum* is not abundant in the regions of the Old Maya Empire in Guatemala and the New Empire in Yucatan. When the Mayas abandoned the Old Empire cities about 600 A. D. (cf. Spinden,¹⁶ Morley¹⁷) they moved in two directions, north to Yucatan and west to the highlands. At least one city in western Guatemala, Quen Santos (cf. Seler¹⁸), is characterized by the same dated monuments which appeared in all the larger Maya cities and the earliest date inscribed at Quen Santos is identical with the date on the lintel at Chichen

Itza, and both are exactly 10 tuns (3600 days) later than the last dates at the two Old Empire cities, Seibal and Tikal. The only place in America where *Euchlaena* has been found growing as the dominant species is in the highlands of western Guatemala not far from the ruins of Quen Santos. *Tripsacum* grows in profusion here; and here, too, is the only region where the natives have a Maya word, *salic* or *salicim*, for *Euchlaena*.⁶ All of these facts suggest, though do not prove, that *Euchlaena* originated after the Mayas abandoned the Old Empire and migrated to the highlands bringing *Zea* and *Tripsacum* into direct contact with each other on a large scale.

With *Euchlaena* eliminated as a recent development, there remains no reason for dismissing pod-corn as the putative ancestor of cultivated maize. In the homozygous state pod-corn is often an earless, perfect-flowered plant bearing its seeds in the terminal inflorescence or tassel.¹⁰ It possesses the two essential characteristics which maize now lacks and which it must have had in order to survive in the wild, a protection for the individual seed and a means of dispersal. The first is provided by prominent glumes, the second by a brittle rachis. Since we have at hand in pod-corn all of the characteristics which the morphologists demand in the progenitor of maize, and since in its homozygous form pod-maize is identical with other cereals, in its brittle rachis, perfect flowers and glume-enclosed seeds, and finally since the very existence of this peculiar form requires an explanation, we see no necessity for searching further for the prototype of maize.

The change from pod-corn to naked corn is the result of a recessive single gene mutation, a mutation which may have occurred repeatedly in the past only to be lost in nature. Once the mutation occurred under domestication, however, it immediately acquired a survival value which it had never previously possessed. Many other changes must have followed this original mutation, one of these being the shortening of the axes of the lateral branches so that the lateral inflorescences are now enclosed in the overlapping leaf sheaths, or shucks. The pod-corn of today, therefore, is the result of superimposing upon a plant, which has been tremendously altered by millennia of domestication, a single relic wild gene, and though modern pod-corn has most of the essential characteristics of the original wild corn, it must differ greatly from the wild plant in many others.

There is also no longer any necessity for seeking the center of origin of maize in Mexico or Central America; most of the evidence, except its close relationship to teosinte, has consistently pointed to Peru as the primary center of domestication. Here appears the first serious discrepancy in the argument, for Peru is one of the few places in America where pod-corn is unknown. This may be accounted for by assuming that pod-corn has survived as a relic gene in the maize varieties of the more primitive Indians, who, notoriously careless in maintaining purity in their varieties, have

never practiced rigid selection against the podded characteristic, but that in Peru where agriculture reached a higher stage of advancement than elsewhere in America, selection finally succeeded in eliminating the podded gene from the population. If this assumption is sound, we might still expect to find pod-corn represented in the prehistoric Peruvian pottery, for the ancient Peruvians depicted many of their food plants on their pottery and some of the representations, particularly those found on the Chimu pottery of the coast, are extremely realistic. Accordingly we began a search in the museums, and among the illustrations in the archaeological literature for representations of pod-corn, a search which reached fruition in the discovery at the Peabody Museum at Yale University of a faithful replica of a prehistoric ear of pod-corn.

Although Peru was undoubtedly the primary center of domestication of maize, it probably was not the original wild habitat, for the geography and climate of Peru are not those under which we should expect maize to have existed as a wild plant. It appears far more likely that maize had its native habitat in the lowlands of South America, not in the tropical rain forests, for maize competes but poorly with tall vegetation and its seed have no mechanism for maintaining dormancy under humid conditions. But the forests of South America are interspersed with rainy-green savannas where an abundance of rain is followed by a dry period.¹⁹ In such regions a wild maize plant might easily persist. Supporting this assumption are found four independent historical references associating pod-corn with the Guarany Indians of South America or with the region which they occupied.^{7,20,21,22} One of these,²¹ describes a type of maize which bears its seeds in the tassel and which may well have been the homozygous, earless form of pod-corn.

There is no evidence in conflict with the view that maize had but one center of origin. In the case of several other crops, notably beans and squashes, it appears that domestication proceeded independently in Peru and Central America, but this is not true of maize. There is, however, rather conclusive evidence that hybridization of *Zea* and *Tripsacum* which produced *Euchlaena*, and subsequent repeated hybridizations of *Euchlaena* with its *Zea* parent, have given rise to new types of maize previously not in existence. We should have expected this on *a priori* grounds and on the same grounds we should conclude that the pointed-seeded pop corns represent one of the products of this hybridization. Kuleshov's²³ studies of the Russian world collection of corn varieties show that the pointed pop corns are almost unknown in Guatemala and Peru, but occur in greater diversity in Mexico than in all of the rest of the world combined. The long slender straight-rowed flint and flour corns, which are also putative products of *Tripsacum* contamination, are also rare in Guatemala or Peru, and in this case also in Mexico, but make their first appearance in the Pueblo region

of the Southwest at about the year 900 A. D. It appears to us that almost all of the North American maize varieties exhibit a *Tripsacum* influence and that the chief differences between North and South American varieties are attributable to an infection of *Tripsacum* genes in the former; its absence in the latter.

This hypothesis suggests a number of genetic and cytological tests, one of the most obvious being a comparison of the knobs on the chromosome of North American and South American varieties. The presence of chromosome knobs is one of the many characteristics in which *Euchlaena* is intermediate between *Zea* and *Tripsacum*.²⁴ If the knobs on the chromosomes of *Euchlaena* were derived originally from *Tripsacum*, some species of which possess knobs on every chromosome, and if knobs on the chromosomes of North American maize varieties were also received originally from *Tripsacum* by way of *Euchlaena*, we might expect the indigenous South American maize varieties to be completely lacking in knobs.

At this writing we have examined the prophase chromosomes of only one South American variety, the well-known giant-seeded Cuzco, a variety which is definitely of South American origin. The chromosomes of Cuzco were found to be completely lacking in knobs. If this proves to be a constant characteristic of South American varieties, it will serve as a useful criterion in distinguishing pure maize from *Tripsacum*-infected varieties and will aid in tracing the movements of maize in America in prehistoric times.

The tripartite hypothesis that cultivated maize had its origin in South America as a single gene mutation from a wild form of pod-corn; that *Euchlaena* is a recent product of the natural hybridization of *Zea* and *Tripsacum* which occurred when the two genera were brought together in Central America, and that new types of maize originating from this cross comprise the majority of North American varieties, is in accord, we believe, with all the known facts. Its value as a working hypothesis has already been established by the discovery of pod-corn in the prehistoric Peruvian pottery and of maize from South America with knobless chromosomes, discoveries which had been predicted on hypothetical grounds. The chief additional evidence which might be demanded, the actual collection of pod-corn in the wild in South America, may also be forthcoming when we begin to seek this plant in the unforested lowlands of Southwestern Brazil, Bolivia or Paraguay.

¹ DeCandolle, *Géographie Botanique*, Paris (1855).

² Darwin, *The Variation of Animals and Plants under Domestication*, London (1868).

³ Laufer, *Congrès Internat. d. Américanists*, 15 [1] 223-257 (1907).

⁴ Knowlton, *Jour. Wash. Acad. Sci.*, 9, 134-136 (1919).

⁵ Brown, *Ibid.*, 24, 293-296 (1934).

⁶ Kempton and Popenoe, *Carnegie Inst. of Wash. Publ.*, 483, 199-218 (1937).

- ⁷ Saint-Hilaire, *Ann. d. Sci. Nat.*, **16**, 143-145 (1829).
- ⁸ Sturtevant, *Bull. Torrey Bot. Club*, **21**, 319-343 (1894).
- ⁹ Harshberger, *Garden and Forest*, **9**, 522-523 (1896).
- ¹⁰ Collins, *Jour. Wash. Acad. Sci.*, **21**, 520-530 (1912).
- ¹¹ Weatherwax, *Amer. Midland Naturalist*, **16**, 1-71 (1935).
- ¹² Spinden, *Proc. 19th Int. Cong. Americanists*, 269-276 (1917).
- ¹³ Emerson and Beadle, *Zeitschr. f. indukt. Abstamm. u. Vererb.*, **62**, 305-315 (1932).
- ¹⁴ Beadle, *Genetics*, **17**, 481-501 (1932).
- ¹⁵ Mangelsdorf and Reeves, *Jour. Hered.*, **22**, 329-343 (1931).
- ¹⁶ Spinden, *A Study of Maya Art*, Cambridge, Mass. (1913).
- ¹⁷ Morley, *Carnegie Inst. of Wash. Publ.*, **219**, 1-643 (1920).
- ¹⁸ Seler, *Die alten Ansiedelungen von Chacula*, Berlin (1901).
- ¹⁹ Weberbauer, *Field Mus. Nat. Hist. Publ.*, **351**, 13-81 (1936).
- ²⁰ Dobrizhoffer, *An Account of the Abipones*, London (1822).
- ²¹ Azara, *Voyages dans l'Amérique Méridionale*, Vol. I, Paris (1809).
- ²² Bonafous, *Histoire naturelle, agricole et économique du Mais*, Paris (1836).
- ²³ Kuleshov, *Jour. Am. Soc. Agron.*, **25**, 688-700 (1933).
- ²⁴ Longley, *Jour. Ag. Res.*, **54**, 835-862 (1937).

CALCULATIONS OF BIOELECTRIC POTENTIALS. III. VARIATION IN PARTITION COEFFICIENTS AND ION MOBILITIES

BY S. E. HILL AND W. J. V. OSTERHOUT

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated June 24, 1938

Very interesting differences are found between the cells of *Nitella flexilis*, Ag., in current use and those employed in earlier experiments.¹ The earlier cells came from a different locality and for convenience we shall call them Lot A and the later ones Lot B.

In the earlier cells Na^+ and K^+ seemed to have very different mobilities in X , the outer non-aqueous protoplasmic surface layer. But in the later cells these mobilities, U_{K} and U_{Na} , are not far apart. We therefore expected much less change in P.D. on replacing NaCl by KCl but were surprised to find that the change is somewhat greater.

This can be accounted for if we assume that in the later cells the partition coefficient² S (concentration in $X \div$ concentration in the external solution) is much greater for KCl than for NaCl.

In the previous studies¹ the P.D. could be satisfactorily predicted by regarding it as due to diffusion potential and hence obeying the equations of Nernst and of Henderson.

The apparent mobility was calculated from the equation³ of Nernst which may be written (for 20°C.)

$$\text{P.D.} = 58 \frac{u - v}{u + v} \log \frac{C_1}{C_2}$$

where C_1 and C_2 are concentrations, u is the mobility of the cation and v that of the anion: for convenience we put $v = 1$ and calculate the value of u .⁴

In Lot A it was found¹ that the replacement of 0.001 M by 0.01 M gave a change of P.D. of 20.9 mv. for NaCl and of 54.7 mv. for KCl. From this we get $u_{\text{Na}} = 2.18$ and $u_{\text{K}} = 73.24$. In Lot B⁵ the corresponding values⁶ are 45 and 49, giving $u_{\text{Na}} = 7.93$ and $u_{\text{K}} = 11.9$.

Using these values we may calculate the changes of P.D. to be expected when 0.01 M NaCl is replaced by 0.01 M KCl (this is called the potassium effect). For this purpose we make use of Henderson's equation. This may be written (for 20°C.)

$$\text{P.D.} = 58 \frac{(U_I - V_I) - (U_{II} - V_{II})}{(U_I + V_I) - (U_{II} + V_{II})} \log \frac{U_I + V_I}{U_{II} + V_{II}}$$

where $U_I = u_{\text{K}}C_{\text{K}}$, $U_{II} = u_{\text{Na}}C_{\text{Na}}$, $V_I = v_{\text{Cl}}C_{\text{K}}$ and $V_{II} = v_{\text{Cl}}C_{\text{Na}}$: all values apply to the protoplasmic surface, X ; u_{K} and u_{Na} denote mobilities and C denotes concentration.

In dealing with Lot A it was assumed that the partition coefficient S was the same for KCl and NaCl so that in X we should have $C_{\text{K}} \div C_{\text{Na}} = 1$. It was then found that the calculated value was not far from the value of 83 mv. observed⁷ on substituting 0.01 M KCl for 0.01 M NaCl.

In Lot B the calculated value on this basis is 9.3 mv. and the observed value 95 ± 2 mv. (120 observations on 44 cells) but if we suppose that S is greater for KCl than for NaCl so that in X we have $C_{\text{K}} \div C_{\text{Na}} = 60$ the calculated value⁸ becomes 95 mv.

It would not be surprising if the partition coefficient for potassium were higher than for sodium since potassium is usually taken up in preference to sodium by living cells. It must be remembered, however, that we are here dealing with the concentration of K^+ and Na^+ in X rather than with that of KCl and NaCl: the latter may be weak electrolytes in X and may have different dissociation constants.⁹

These calculations indicate that the partition coefficient can vary greatly and this seems highly probable in view of experiments with models which show that the partition coefficients of organic and inorganic substances between aqueous and non-aqueous phases can be greatly influenced by the addition of small amounts of reagents.¹⁰ If the protoplasmic surface layer is non-aqueous, as we suppose, it is easy to see how great changes in partition coefficients might occur as the result of metabolism.

Not only the partition coefficients but also the mobilities differ from those found in Lot A. In Lot A we have $u_{\text{K}} = 73.24$, $u_{\text{Na}} = 2.18$; in Lot B,

$u_K = 11.9$ and $u_{Na} = 7.93$. Such differences¹¹ are not surprising in view of the fact that the removal of organic substances from the cell by distilled water can reduce¹² u_K from 85 to 2 or less: this can be restored by a variety of substances.¹³ Changes can also be produced by guaiacol.¹⁴ Hence it seems possible that alterations in metabolism can affect mobilities as well as partition coefficients.

Summary.—From changes in p.d. produced by changes of concentration we can calculate the apparent mobilities of K^+ and Na^+ in the outer protoplasmic surface of *Nitella*. We can thus predict the potassium effect (the change in p.d. produced by substituting 0.01 *M* KCl for 0.01 *M* NaCl) by assuming a value for the ratio of partition coefficients $S_{KCl} \div S_{NaCl}$ (S = concentration in the outer non-aqueous protoplasmic surface layer \div concentration in the external solution).

In cells studied formerly it was assumed that $S_{KCl} \div S_{NaCl} = 1$ but in the cells used in the present investigation we assume that $S_{KCl} \div S_{NaCl} = 60$.

Such variations in S do not appear improbable in view of experiments on models.

In the cells studied earlier the apparent mobilities of K^+ and Na^+ differed from those found in the present investigation. This is not surprising as alterations of mobilities can be brought about by a variety of reagents and the apparent mobilities might therefore be expected to vary according to the metabolism of the cell.

¹ Cf. Osterhout, W. J. V., *Jour. Gen. Physiol.*, **13**, 715 (1929–1930).

² The term partition coefficient is used in liberal sense. If we have KCl in the external solution K^+ in X may be to some extent paired with organic anions or may form complexes in the sense of Kraus and Fuoss (Kraus, C. A., *Trans. Electrochem. Soc.*, **66**, 179 (1934); Fuoss, R. M., *Chem. Rev.*, **17**, 27 (1935)).

³ It is assumed that the partition coefficient, S , is constant which is probably not far from true in this case. In models S is relatively constant for NaCl and KCl as compared with sodium guaiacolate and potassium guaiacolate. Concentrations are employed for convenience in place of activities.

⁴ All values relate to X .

⁵ For the technique employed in Lot B, see Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **21**, 541 (1937–1938). All measurements were made from photographic records.

⁶ For NaCl 45 ± 0.69 mv. (75 observations on 37 cells) and for KCl 49 ± 0.49 mv. (95 observations on 41 cells).

⁷ In all cases KCl is negative to NaCl in the external circuit.

⁸ For convenience we put $C_{Na} = 1$, $V_{Cl} = 1$. We then have $U_I = 11.9 (60) = 714$, $V_I = (1) 60 = 60$, $U_{II} = 7.93 (1) = 7.93$ and $V_{II} = 1 (1) = 1$.

⁹ We suppose that X is a non-aqueous layer with a low dissociation constant (Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 967 (1933); *Trans. Faraday Soc.*, **33**, 997 (1937); Shedlovsky, T., and Uhlig, H. H., *Jour. Gen. Physiol.*, **17**, 549, 563 (1933–1934)).

¹⁰ Unpublished. See also Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **21**, 553 (1937–1938) (footnote 29).

¹¹ These mobilities when determined from concentration effects are largely independent of partition coefficients. See footnote 3.

¹² Osterhout, W. J. V., *Jour. Gen. Physiol.*, **18**, 992 (1934-1935).

¹³ Cf. Osterhout, W. J. V., and Hill, S. E., *Proc. Soc. Exptl. Biol. and Med.*, **32**, 715 (1934-1935).

¹⁴ Unpublished. For changes in mobilities produced by guaiacol in *Valonia*, see Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20**, 13, 685 (1936-1937).

RAYLEIGH WAVES

BY H. BATEMAN

NORMAN BRIDGE LABORATORY OF PHYSICS, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated June 18, 1938

1. *The Interaction of the Air and the Ground.*—The transmission of plane longitudinal waves of unlimited extent from the ground to the air was investigated by C. G. Knott¹ many years ago. He found that the resulting air waves, which are propagated in almost a vertical direction, generally have only a small amount of energy. Earthquake sounds have been studied by many writers. C. Davison² put forward the theory that they originate from the margin of the region disturbed by the earthquake and travel some distance through the earth before being transmitted to the air. A summary of results relating to earthquake noises has been given by Landsberg.³ The type of air motion considered here is not the simple progressive wave in an unlimited atmosphere but is a type of free vibration of the air and ground having the characteristics of a Rayleigh wave except that its velocity of propagation is less than the velocity of sound in air instead of being slightly less than the velocity of a shear wave in the ground. The mathematical analysis is very similar to that used by Stoneley⁴ in his study of Rayleigh waves in a plane homogeneous elastic earth below a compressible sheet of water of unlimited extent. It is assumed here, however, that the vertical velocity of the air is negligible at a height H above the ground while in Stoneley's work the boundary condition at the free surface of the water is one of constant pressure. His remarks on nodal planes indicate that his analysis may be applicable in our case but it has been thought worth while to give the analysis again in a form in which the velocity of the wind is taken into consideration and some of Stoneley's approximations are omitted. It is thought that the analysis may be of some interest in connection with the interpretation of the ground roll observed in geophysical field work. For information relating to the ground roll I am indebted to Dr. Gutenberg, Mr. Martin Gould and other members of the group connected with the Pasadena Seismological Laboratory. It

has generally been assumed, of course, that the influence of the air on the propagation of seismic waves is slight but such an assumption ought to be justified by numerical work in the case of waves produced by an artificial explosion for there are some features of the phenomena that are not fully elucidated. The problem resembles that of the loud speaker with infinite baffle, the disturbed area of the earth corresponding to the membrane that is set in vibration. Now in the theory of the loud speaker the short circuiting of energy is a familiar phenomenon, there is not simply a radiation of energy outwards. If, then, there is a similar short circuiting of energy in the air after an explosion, an interaction of air and ground is to be expected. If the air and ground are treated as a coupled system, an explosion may be expected to give rise to a subsequent motion that is composed of free vibrations of the system and the particular type of motion to be studied is, indeed, a free vibration. The whole problem is, then, one of the partition of energy among a number of free vibrations including in particular the ordinary Rayleigh wave and the new type of Rayleigh wave. This second type of Rayleigh wave is called "new" merely to distinguish it from the old type but it has been known for a long time that there is more than one type of Rayleigh wave for a stratified medium. With regard to the likelihood of the existence of a marked interaction between the earth and the air it should be mentioned that many years ago the late Lord Rayleigh⁵ concluded that in the vicinity of a vibrating body of linear dimensions small in comparison with the wave-length, the air acts as if it were almost incompressible while the great mass of air at some distance from the body is slightly compressed periodically. A similar conclusion has been reached more recently by Lennard Jones⁶ after some elaborate calculations. In trying to apply this result to our problem we are led to surmise that when the ground rises initially after an explosion the air immediately above it will either move away laterally and produce a reaction on the ground somewhere else or will try to lift or compress the great body of air above it.

2. *The Problem of a Flat Earth and a Homogeneous Atmosphere.*—Taking the axis of x along the ground in the direction of propagation and the axis of z vertically downward, the component displacements, u , w , in the earth, which for simplicity is supposed to be homogeneous, can, as in the theory of the late Lord Rayleigh,⁷ be expressed in the form

$$u = u_1 + u_2, \quad w = w_1 + w_2,$$

where

$$u_1 = ifPe^{-rs} - ifX, \quad w_1 = rPe^{-rs} - ifX$$

$$u_2 = isQe^{-sz} - ifX, \quad w_2 = fQe^{-sz} - ifX$$

$$X = x - vt, \quad v = \text{velocity of propagation,}$$

$$r^2 = f^2(1 - v^2/a^2) = f^2C^2(v), \text{ say,}$$

$$s^2 = f^2(1 - v^2/b^2) = f^2S^2(v), \text{ say.}$$

The symbol a is used here to denote the velocity of propagation of longitudinal waves and the symbol b to denote the velocity of propagation of waves of shear.

Supposing for simplicity that the undisturbed air has a velocity V (independent of z , x and t) parallel to the axis of X , a constant density ρ and a constant velocity of sound c , the velocity potential, ϕ , of a small irrotational perturbation satisfies the partial differential equation

$$c^2 \left(\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial z^2} \right) = \left(\frac{\partial}{\partial t} + V \frac{\partial}{\partial x} \right)^2 \phi.$$

This equation has a solution of the form

$$\phi = A e^{-ms - ifX} + B e^{ms - ifX}$$

in which A , B , m are constants, if m is given by the equation

$$m^2 = f^2 [1 - V^2/c^2] = f^2 M^2(v), \text{ say.}$$

When $(v - V)^2 < c^2$, m is real and the air wave may be regarded as of Rayleigh's type. When $(v - V)^2 > c^2$, the air wave consists of a wave of sound and its reflection at some reflecting layer.

The component velocities of the air are U , W , where

$$U = \frac{\partial \phi}{\partial x} = -if(A e^{-ms} + B e^{ms}) e^{-ifX}$$

$$W = \frac{\partial \phi}{\partial z} = m(B e^{ms} - A e^{-ms}) e^{-ifX}.$$

The condition $W = 0$ when $z = -H$ gives $A = K e^{-mH}$, $B = K e^m$, where K is a quantity which may be found from the condition that

$$W = \frac{\partial w}{\partial t} \text{ when } z = 0.$$

Thus

$$2mK \operatorname{sh}(mH) = ifv(rp + fQ).$$

When the viscous drag of the air on the ground is neglected the absence of a shearing traction on the ground gives the equation

$$2frP + (s^2 + f^2)Q = 0.$$

Since the normal excess pressure of the air on the ground due to the perturbed air motion is wholly responsible for the normal traction on the ground, we find that

$$\sigma a^2 \left(\frac{\partial u}{\partial x} + \frac{\partial w}{\partial z} \right) - 2\sigma b^2 \frac{\partial u}{\partial x} = \rho \left(\frac{\partial \phi}{\partial t} + V \frac{\partial \phi}{\partial x} \right)$$

where σ is the density of the ground. Hence

$$\sigma a^2(f^2 - r^2)P - 2\sigma b^2f(fP + sQ) = 2Kif\rho(v - V) \operatorname{ch}(mH).$$

Substituting the values already found for the ratios of P , Q , K , we obtain the equation

$$\sigma b^4 M(v) \tanh [fHM(v)] R(v) = \rho v^3(v - V) C(v) \quad (\text{A})$$

where

$$R(v) = 4S(v)C(v) - [1 + S^2(v)]^2.$$

This equation may be discussed in much the same way as Stoneley's equation. For our present purpose we remark first that when $v < c + V$ and both quantities are less than the velocity v_1 of the ordinary Rayleigh wave which makes $R(v_1) = 0$, then $R(v) > 0$ and if also $v > V$ both sides of equation (A) are positive. If, moreover, v is chosen so that

$$\sigma b^4 M(v) R(v) > \rho v^3(v - V) C(v)$$

there is a value of $\tanh [fHM(v)]$ equal to a positive proper fraction for which equation (A) is satisfied and so H can be found uniquely when v is given. The critical value of v is that given by the equation

$$\sigma b^4 M(v) R(v) = \rho v^3(v - V) C(v).$$

With ordinary values for the elastic constants of the ground this critical value v_c is only slightly less than $c + V$. When $v < v_c$ there is a Rayleigh wave in the ground as well as in the air.

Since r and s decrease as v increases from 0 to b , the amplitudes of the constituents of a Rayleigh wave in the ground drop more rapidly with increasing depth when v is close to c than when v is close to v_1 . The drop in the amplitude of the Rayleigh wave in the air is not quite exponential for

$$U = -2ifK \operatorname{ch}(mz + mH) e^{-ifx}, W = 2mK \operatorname{sh}(mz + mH) e^{-ifx}.$$

When $c + V < v < v_1$ and $N^2(v) = (v - V)^2/c^2 - 1$, the equation for H may be written in the form

$$\sigma b^4 N(v) \tan [fHN(v)] R(v) = -\rho v^3(v - V) C(v). \quad (\text{B})$$

When v is assigned $\tan [fHN(v)]$ has a definite sign when the foregoing equation is satisfied and so the height H of the reflecting layer is confined to certain ranges that are equally spaced. This may mean that a value of v for which $c + V < v < v_1$ can only exist under certain atmospheric conditions. To find these it will be necessary to assume that the temperature and wind velocity vary with altitude. The equation for ϕ is then much harder to solve.

The existence of a large rock or mountain from which air waves can be reflected may also be influential in determining the possible values of v but then the problem of propagation must be studied in three dimensions.

3. *Remarks on the Theory of the Ground Roll.*—Though some seismolo-

gists think that the ground roll has some of the characteristics of a Rayleigh wave yet another explanation has been sought on account of the low velocity of the ground roll as compared with that of the ordinary Rayleigh wave. The existence of a second type of Rayleigh wave which arises from a marked interaction between the air and the earth puts a different complexion on the matter. The part which this type of free vibration plays can only be judged by a study of the waves produced by a shock of short duration. An extension of the work of Lamb,⁸ Banerji,⁹ Coulomb¹⁰ and the Japanese seismologists¹¹ is necessary. The work of Jeans,¹² in which seismic waves are regarded as free vibrations (of high order) of a spherical earth must also be extended. Such extensions may be useful for a study of Stoneley's problem and for a study of the effect of a swiftly moving river on the propagation of Rayleigh waves.

The results of Lamb's calculations are only partly supported by the observations.¹³ Andreotti,¹⁴ who has used them in his study of the microseisms produced by waves of the Adriatic dashing against the coast, thinks that there is good agreement. His numerical calculations are based, however, on the assumption that the whole coast is affected by a wave in one phase all along its length. When, moreover, waves dash against a cliff, diffraction effects must be taken into consideration; the problem is not simply one of a solid with plane face. It is possible, however, that edge effects can be taken into consideration in the simple problem by using surface singularities represented by wave-potentials of type

$$(x + iz)^{-1/2} F(t - \omega/a),$$

where $\omega^2 = x^2 + z^2$. In this connection it is interesting to note that a relativity transformation gives a corresponding solution for a similar type of source moving with velocity v and an integration over a set of such moving sources, with an appropriate choice of the individual functions F , leads to the wave-function

$$\int_{-\infty}^{\infty} [z + 1s - i(x - vt)(1 - v^2/a^2)]^{-1/2} e^{-iks} ds,$$

which, when evaluated, leads to just the type of wave-function used in the representation of Rayleigh waves in two dimensions.

The study of elastic waves in two dimensions is complicated by the lack of simplicity in the solution for a line source. Various forms of the principle of Huygens have been found by Volterra,¹⁵ Picht¹⁶ and others, but have not been used very often. Picht¹⁷ has, however, tried to use the principle of Huygens to interpret theoretically the boundary wave which in a stratified earth, travels with the velocity of the upper medium. It is assumed that the strongly damped wave issuing from the focus sets the boundary between the two media into vibration. Such an assumption is very similar to our

assumption that an explosion in the earth sets the boundary between air and earth into vibration producing a wave which travels with a velocity close to that of the upper medium (air). Dr. Gutenberg has furnished me with the information that the velocity of the ground roll is sometimes greater than the velocity of sound in air but is usually between 100 meters a second and 500 meters a second. The amplitude of the ground roll decreases when the depth of the source increases, being small when the depth is 10 meters and negligible when the depth is 20 meters or more. Banerji's calculations indicate a marked decrease in amplitude of the ordinary Rayleigh wave when the depth of the source increases and we might expect by analogy that there would be a similar decrease of amplitude for the Rayleigh wave of low speed. An attempt must therefore be made to test this surmise. Banerji's calculations were for the three dimensional case to which we must now turn. Here there is the advantage that there is a simple wave function for a point source but much analytical ground work is needed before a complete solution of a problem can be obtained. Coulomb¹⁰ has made some progress by endeavoring to extend Boussinesq's solution of statical elastic problems to wave problems. Much of the analysis depends on the properties of a certain transcendental function which he has studied in some detail. Some further properties of this function are developed in an accompanying paper.

¹ C. G. Knott, *Phil. Mag.* (5) **48**, 64-97 (1899).

² C. Davison, *Ibid.* (5) **49**, 31-70 (1900).

³ H. Landsberg, *Trans. American Geophysical Union*, Part 1, 118-120 (1937).

⁴ R. Stoneley, *Monthly Notices Royal Astronomical Soc., Geophys. Suppl.*, **1**, 349-356 (1926).

⁵ Lord Rayleigh, *Theory of Sound*, Vol. 2, p. 158; *Sc. Papers*, Vol. 4, No. 230.

⁶ J. E. Lennard Jones, *Proc. London Math. Soc.* (2) **20**, 347-364 (1920).

⁷ Lord Rayleigh, *Ibid.* (1) **17**, 4-11 (1885); *Sc. Papers*, Vol. 2, p. 441.

⁸ H. Lamb, *Phil. Trans. Roy. Soc. London (A)* **203**, 1-42 (1904).

⁹ S. K. Banerji, *Phil. Mag.* (6) **49**, 65-80 (1925).

¹⁰ J. Coulomb, *Am. de Toulouse* (3) **23**, 91-137 (1931).

¹¹ H. Nakano, *Japanese Jour. of Astronomy and Geophysics*, **2**, 233-326 (1925); *Geophysical Mag.*, **1**, 255-303 (1926-1928); **2**, 189-348 (1930); H. Arakawa, *Geophysical Mag.*, **7**, 155-160 (1933); K. Sezawa and G. Nishimura, *Bull. Earthquake Research Institute Tokyo Imp. Univ.*, **7**, 41-64 (1929).

¹² J. H. Jeans, *Proc. Roy. Soc. London (A)* **102**, 554-574 (1923).

¹³ J. B. Macelwane, *Bull. National Research Council*, No. 90, 121-128 (1933).

¹⁴ G. Andreotti, *Veneto Atti*, **91**, 1345-1358 (1932).

¹⁵ V. Volterra, *Acta Math.*, **18**, 161-232 (1894).

¹⁶ J. Picht, *Zeit. f. Physik*, **91**, 717-723 (1934).

¹⁷ J. Picht, *Ann. d. Physik* (5) **19**, 913-920 (1934); *Beiträge Angew. Geophysik*, **3**, 1-8 (1933).

COULOMB'S FUNCTION

BY H. BATEMAN

NORMAN BRIDGE LABORATORY OF PHYSICS, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated June 18, 1938

1. In his work on Rayleigh waves Coulomb¹ has studied the function

$$\psi_n(hw, hw \operatorname{sh} a) = i^n \int_a^\infty e^{-ihw \operatorname{ch} u} \operatorname{ch}(nu) du. \quad (1.1)$$

$$\operatorname{ch} a = \cosh a, \operatorname{sh} a = \sinh a$$

The function ψ_0 with a complex value of h occurs in the work of Buchholz² on the propagation of alternating currents in the earth between two electrodes connected above ground by a rectangular loop of wire whose vertical ends support the horizontal piece. Use will be made here of the notation

$$C_n(a, x) = \int_a^\infty e^{-x \operatorname{ch} u} \operatorname{ch}(nu) du, S_n(a, x) = \int_a^\infty e^{-x \operatorname{sh} u} \operatorname{ch}(nu) du \quad (1.2)$$

wherein $R(x) > 0$ and $a > 0$. When $n = 0$ expansions of these functions are readily obtained by putting $x \operatorname{ch} u = v$, $x \operatorname{ch} a = c$ in the first integral and $x \operatorname{sh} u = w$, $x \operatorname{sh} a = s$ in the second. With the notation $(m/, n)$ for the binomial coefficient $C_{m,n}$ and the notation

$$Q(z, k) = \int_z^\infty e^{-t} t^{k-1} dt \quad (1.3)$$

for the incomplete Gamma function of the second kind, the expansions obtained by using the binominal theorem are

$$C_0(a, x) = \sum_{n=0}^{\infty} (-)^n (-1/2/, n) x^{2n} Q(c, -2n) \quad (1.4)$$

$$S_0(a, x) = \sum_{n=0}^{\infty} (-1/2/, n) x^{2n} Q(s, -2n).$$

The first of these expansions is given by Coulomb and Buchholz. The convergence of the series may be established by using the formula

$$Q(c, -m) = e^{-c} c^{-m} / m - e^{-c} c^{-m+1} / m(m-1) + \dots (-)^m Q(c, 0) / m!$$

The second series converges absolutely when $\operatorname{sh} a > 1$. When each term is transformed by using the formula

$$\Gamma(2n+1) e^s Q(s, -2n) = \int_0^\infty e^{-t} t^{2n} dt / (1+t)$$

as in Buchholz's transformation of the series for $C_0(a, x)$, we find that

$$S_0(a, x) = \int_0^\infty \exp [-(1+t)x \operatorname{sh} a] J_0(xt) dt/(1+t), \quad (1.5)$$

while the corresponding formula of Buchholz is

$$C_0(a, x) = \int_0^\infty \exp [-(1+t)x \operatorname{ch} a] I_0(xt) dt/(1+t). \quad (1.6)$$

It should be noticed that by expanding $1/(1+t)$ in powers of t and integrating term by term we obtain the same asymptotic series for $S_0(a, x)$ as is obtained from (1.2) by repeated integration by parts.

A relation between $C_n(a, x)$ and $S_n(a, x)$ may be found by putting $s = \operatorname{sh} u$ in the integral

$$\int_0^\infty e^{-sz} J_0[(z^2 + 2xz)^{1/2}] dz = (1+s^2)^{-1/2} \exp[-x\{(1+s^2)^{1/2} - s\}], \quad (1.7)$$

multiplying by $\operatorname{ch} nu e^{-x \operatorname{sh} u} du$ and integrating from a to ∞ . This gives

$$\begin{aligned} C_n(a, x) &= -(d/dx) \int_a^\infty e^{-x \operatorname{ch} u} \operatorname{ch}(nu) du / \operatorname{ch} u \\ &= -(d/dx) \int_0^\infty S_n(a, x \operatorname{sh} a \operatorname{ch} v) J_0(x \operatorname{sh} v) x \operatorname{sh} v dv. \end{aligned} \quad (1.8)$$

If, on the other hand, we multiply (1.7) by $e^{-x \operatorname{sh} u} \operatorname{ch} u du$ and integrate from a to ∞ we find that

$$C_0(a, x) = \int_0^\infty e^{-x \operatorname{ch} v \operatorname{sh} a} J_0(x \operatorname{sh} v) \tanh v dv. \quad (1.9)$$

2. Another expansion for $C_0(a, x)$ may be found by using the function

$$V_n(x) = \int_0^\infty e^{-xt} P_n\left(\frac{t-1}{t+1}\right) dt/(t+1) = e^x \int_x^\infty e^{-u} P_n(1-2x/u) du/u \quad (2.1)$$

which has been studied in a former paper.³ If $V_n(x) = e^x W_n(x)$ there is an expansion

$$W_n(x) = \sum_{m=0}^n (-n/, m)(n+m/, m) x^m Q(x, -m) \quad (2.2)$$

a differential equation

$$x^2 W_n'' + (x^2 + 3x) W_n' + (2x + 1) W_n - n(n+1) W_n = 0 \quad (2.3)$$

and recurrence relations

$$x(W_n' + W_{n-1}') = n(W_n - W_{n-1}).$$

$$(n+1)W_{n+1}' + nW_{n-1}' - (2n+1)W_n' = 2(2n+1)W_n.$$

$$\begin{aligned}
(4n+2)x(W'_n + W_n) &= (n+1)^2(W_{n+1} - W_n) + n^2(W_n - W_{n-1}), \\
x(W''_{n+1} - W''_{n-1}) &= 2(2n+1)W_n + W'_{n-1} - W'_{n+1}, \\
n^2W_{n-1} &= 2x^2W''_n + [2x^2 - 2(n-1)x]W'_n - n(2x-n)W_n \\
(n+1)^2W_{n+1} &= 2x^2W''_n + [2x^2 + 2(n+2)x]W'_n \\
&\quad + (n+1)(2x+n+1)W_n \\
(2n-1)(n+1)^2(W_{n+1} - W_n) &- 2n(2n^2-1)(W_n - W_{n-1}) \\
+ (n-1)^2(2n+1)(W_{n-1} - W_{n-2}) &= 2x(4n^2-1)(W_n + W_{n-1}). \quad (2.4)
\end{aligned}$$

The differential equation for $W_n(x)$ is adjoint to the differential equation

$$x^2Z'''_n + (3x - x^2)Z''_n + (1 - 2x)Z'_n + n(n+1)Z_n = 0, \quad (2.5)$$

which is satisfied by the function $Z_n(x) = F(-n, n+1; 1, 1; x)$ which was studied at the same time³ as $V_n(x)$. This function $Z_n(x)$ may be used to obtain the representation

$$V_n(z) = \lim_{x \rightarrow 1} Z_n(-d/dx)V_0(zx). \quad (2.6)$$

The generating function of $W_n(x)$ suggests the expansion

$$\int_a^\infty e^{-z \operatorname{ch} u} du = (1 - e^{-a}) \sum_{n=0}^\infty e^{-na} W_n[z(\operatorname{ch} a - 1)] \quad (2.7)$$

which is certainly convergent when $z > 0$, $a > 0$ but may be valid under more general conditions.

3. In the physical investigations the wave potential connected with $C_0(a, x)$ $C(a, x)$ is

$$W = \int_s^\infty e^{-kR} ds/R, \quad (3.1)$$

where k is a complex constant and $R^2 = s^2 + w^2 = s^2 + x^2 + y^2$, x , y and z being rectangular coördinates. With $s = w \operatorname{sh} u$, $z = w \operatorname{sh} a$ the integral is $C_0(a, kw)$ and if $r^2 = z^2 + w^2 = x^2 + y^2 + z^2 = w^2 \operatorname{ch}^2 a$ the expansion of the integral W is

$$\begin{aligned}
W &= e^{-kw} (1 - e^{-a}) \sum_{n=0}^\infty e^{-na} W_n(kr - kw) \\
&= e^{-kw} \sum_{n=0}^\infty [(r-z)/w]^n [W_n(kr - kw) - W_{n-1}(kr - kw)] \quad z > 0
\end{aligned} \quad (3.2)$$

where it is understood that $W_{-1}(x) \equiv 0$. It is thought that this expansion will converge rapidly. This surmise can be checked as soon as the tables of the function $W_n(x)$ have been completed.

If $v = w (\text{ch } a - 1) = r - w$, there is also an expansion

$$\int_0^\infty e^{-kw \text{ch } u} du = \sum_{n=0}^\infty (-1/2, n) (2w)^{2n} Q(kv, -n). \quad (3.3)$$

4. There is an integral relation

$$\int_0^\infty t^{s-1} W_n(t) dt = \Gamma(s) G_n(s) \quad R(s) > 0 \quad (4.1)$$

in which

$$s(1+s)(2+s)\dots(n+s)G_n(s) = (1-s)(2-s)\dots(n-s). \quad (4.2)$$

This is readily derived from (2.2) and suggests the new definition

$$W_n(t) = (1/2\pi i) \int_{c-i\infty}^{c+i\infty} t^{-s} \Gamma(s) G_n(s) ds \quad (4.3)$$

which, when $c > 0$, may be found directly by an attempt to solve the differential equation by means of a definite integral. The function $G_n(s)$ occurs as a coefficient in the expansion

$$(\text{ch } a - 1)^s \int_a^\infty (\text{ch } u - 1)^{-s} du = (1 - e^{-a}) \sum_{n=0}^\infty e^{-na} G_n(s). \quad 0 < s < 1 \quad (4.4)$$

5. Another representation of $W_n(x)$ which may be useful in finding new properties of the function is

$$W_n(x) = (\pi/x)^{1/2} \int_0^\infty J_0(u) I_{n+1/2}(u^2/8x) \exp(-u^2/8x) du. \quad (5.1)$$

This is valid so long as $R(x) > 0$. The formula may be checked by means of the recurrence formulae for $W_n(x)$ and the finite series for $I_{n+1/2}(z)$.

6. An asymptotic expansion for $V_n(x)$ for large values of z such that $R(z) > 0$ may be obtained from the series

$$V_n(z) = \sum_{m=0}^n (-n/, m)(n + m/, m) \int_0^\infty e^{-zt} (t+1)^{-m-1} dt \quad (6.1)$$

by using the asymptotic expansion of each of the integrals, it is

$$V_n(z) \sim \sum_{r=0}^\infty \sum_{m=0}^n (-n/, m)(n + m/, m)(-m - 1/, r) z^{-r-1} r! = \sum_{r=0}^\infty F_n(2r+1) z^{-r-1} (-)^r \quad (6.2)$$

where $F_n(x)$ is the polynomial studied in a former paper.⁴

7. It follows at once from the formula

$$V_n(x) = \int_0^\infty e^{-t} Z_n(t) dt / (x + t) \quad (7.1)$$

that

$$V_n(x) = V_0(x)Z_n(-x) + p_{n-1}(x) + p_{n-2}(x) + \dots + p_0(x), \quad (7.2)$$

where $p_{n-1}(x)$ is a polynomial of degree $n - 1$ in x . Hence as $x \rightarrow 0$ $V_n(x) - V_{n-1}(x) \rightarrow p_{n-1}(0)$. To verify that this is equal to $-2/n$ we may use the last recurrence formula (2.4), this value being readily found when $n = 1$ and $n = 2$. This leads to the formula

$$\lim_{x \rightarrow 0} [V_0(x) - V_n(x)] = 2 \left(1 + \frac{1}{2} + \dots + \frac{1}{n} \right). \quad (7.3)$$

On account of this relation it is often convenient to transform a series of type $\sum c_n V_n(x)$ into one of type

$$\sum_{n=0}^{\infty} b_n [V_n(x) - V_{n-1}(x)]$$

on the understanding that $V_{-1}(x) = 0$. The convergence of the resulting series as $n \rightarrow \infty$ may then be readily tested. In particular it is found that the series (3.2) fails to converge when $z = 0$ as is to be expected.

¹ J. Coulomb, *Annales de Toulouse* (3), **23**, 91-137 (1931).

² H. Buchholz, *Arkiv für Elektrotechnik*, **30**, 1-33 (1936).

³ H. Bateman, *Duke Math. Jour.*, **2**, 569-577 (1936).

⁴ H. Bateman, *Tôhoku Math. Jour.*, **37**, 23-38 (1933).

⁵ S. O. Rice, *Bell System Technical Jour.*, **16**, 101-109 (1937).

⁶ H. Bateman and S. O. Rice, *Amer. Jour. Math.*, **60**, 297-308 (1938).

⁷ The function $C_0(a, x)$ occurs also in a paper by S. O. Rice⁵ in which a transformation is given of van der Pol's expression for the value on the ground of the wave-function of a vertical dipole placed at the surface of a plane earth. Expansion for $C_0(a, x)$, $C_n(a, x)$ may also be found by using the integrals (14) and (15) in a recent paper by the author and S. O. Rice.⁶

FUNCTIONAL TOPOLOGY AND ABSTRACT VARIATIONAL THEORY

BY MARSTON MORSE

INSTITUTE FOR ADVANCED STUDY, PRINCETON, N. J.

Communicated June 28, 1938

This paper is concerned with a summary of results which will appear at length in a fascicle in the series "Mémoires des sciences mathématiques," Gauthier-Villars, Paris. Part of the theory is based on certain conceptions and theorems in group theory. These will be summarized first in order to make clear their independence from the rest of the theory.

1. *The Group Theory.*—Let G be an additive abelian operator group with coefficients δ in a field Δ . With certain of the elements u of G we associate an element $\rho(u)$ in a simply ordered set of elements $[\rho]$. The set $[\rho]$ may in particular be the set of real numbers. We term $\rho(u)$ the *rank* of u . The rank $\rho(0)$ shall not be defined. The elements of G with rank (with 0 added) will not in general form a group. A subgroup g of G will be termed an *operator subgroup* if when u is in g , δu is in g . The property A is termed an *operator property* if whenever u has the property A and $\delta \neq 0$, δu has the property A . By a subgroup g of G with property A is meant an operator subgroup every element of which with the possible exception of 0 has the property A . The group g will be termed *maximal* if it is a proper subgroup of no subgroup of G with property A . The ranks $\rho(u)$ shall satisfy the following three conditions:

I—If u has a rank and $\delta \neq 0$, $\rho(u) = \rho(\delta u)$

II—If u , v , and $u + v$ have ranks, then

$$\rho(u + v) \leq \max [\rho(u), \rho(v)]$$

III—If u and v have unequal ranks $\rho(u + v)$ exists.

The proof of the following theorem is due to R. Baer.

THEOREM 1.1. *Let g be an operator subgroup of G whose dimension is at most alef-null. If each element of g save the null element has a rank, g is a direct sum of suitably chosen maximal subgroups $g(\rho)$ of elements of g with the respective ranks ρ .*

The author has proved a similar theorem in the case where the ranks taken in their natural order are well ordered. Without some restriction on g a theorem of this sort cannot be proved. A fourth condition on the ranks is met in practice.

IV—If u_1, \dots, u_m and v_1, \dots, v_n are elements of G with ranks at most a_0 while the sums $u = \sum u_i$ and $v = \sum v_j$ have no rank and $u + v$ has a rank, then $\rho(u + v) < a_0$.

We shall say that two elements u and v of G are in the same *rank class*

if u and v have the same rank while $u - v$ has no rank or a lesser rank. An isomorphism between two subgroups of G of elements with rank will be termed a *rank isomorphism* if corresponding non-null elements are in the same rank class.

THEOREM 1.2. *When conditions I to IV are satisfied any two maximal subgroups of elements of G with the same rank are rank isomorphic.*

This theorem enables us to assign type groups and type numbers to critical sets.

2. *The Space M and Function F .*—Let M be a metric space of points p, q , etc. Let $F(p)$ be a real single-valued function of the point p on M , with $0 \leq F \leq 1$. By the set $F \leq b$ is meant the subset of points of M at which $F(p) \leq b$. Let U be an homology class with elements which are non-bounding k -cycles u . If u is on $F \leq b$, b will be called a *cycle bound* of u and of U . The greatest lower bound of the cycle bounds of U will be called the *cycle limit* $s(u)$ of U and of the elements u of U . If U is the class of bounding k -cycles $s(u)$ will not be defined. Let G be the group of all k -cycles. With some but not all of the elements u of G we have thus associated a number $s(u)$. We term $s(u)$ the rank of u . These ranks satisfy the four rank conditions of §1. Hence the theorems of §1 hold with the present interpretation. Theorem 1.1 has here the following corollary, considerably weaker than the theorem.

COROLLARY. *The sum of the dimensions of maximal groups, $g(s)$, of non-bounding k -cycles with the respective cycle limits s is at least the smaller of the two numbers alef-null and the k th connectivity R_k of M .*

Up to the present point it has been immaterial whether ordinary singular cycles are understood or Vietoris cycles. From this point on we shall refer to Vietoris cycles. See M., §2.¹ We shall now state the first of two fundamental hypotheses, that of *F-accessibility*. If Vietoris cycles are used this hypothesis is fulfilled in the ordinary variational theory. It is not in general fulfilled if ordinary singular cycles are used.²

Under the hypothesis of F-accessibility any non-bounding k -cycle which is $\sim 0 \bmod F < c + \epsilon$ for each positive ϵ , is homologous to a k -cycle on $F \leq c$.

A non-bounding k -cycle v whose rank is $s(v)$ and which is on $F \leq s(v)$, will be termed *canonical*. Under the hypothesis of *F-accessibility* there is at least one canonical k -cycle in each non-null homology class. If the sets $F \leq c$ are compact for each $c < 1$ the hypothesis of *F-accessibility* is satisfied, as we prove.

k -Caps. A point set A will be said to be *definitely below* a (written d-below a) if A lies on $F < a - \epsilon$ for some positive ϵ . The phrase d-mod $F < a$ shall be understood to mean mod some compact set d-below a . If u is a k -cycle on $F \leq a$, d-mod $F < a$, an homology

$$u \sim 0$$

$$(\text{on } F \leq a, \text{ d-mod } F < a)$$

will be called an *a-homology*. A k -cycle u , d-mod $F < a$ on $F \leq a$, not a -homologous to zero, will be called a k -cap with cap limit a . We write $a = a(u)$. These cap-limits satisfy the four rank conditions of §1.

THEOREM 2.1. *Under the hypothesis of F -accessibility a canonical non-bounding k -cycle u with cycle limit $s(u)$ is a k -cap with cap limit $s(u)$.*

Let p be a point of M at which $F(p) = c$. The set M will be said to be *locally F -connected of order $m > 0$* at p if corresponding to each positive constant ϵ there exists a positive constant δ such that each singular $(m-1)$ -sphere on the δ -neighborhood of p and on $F \leq c + \delta$ bounds an m -cell of diameter less than ϵ on $F \leq c + \epsilon$. If each subset $F \leq c < 1$ of M is compact and if M is locally F -connected of all orders from 1 to $m+1$ at points of $F < 1$, then the dimension of the m th homology group of $F < 1$ is at most alef-null, and the cycle limits $s(u)$ have at most the cluster value 1.

3. *Homotopic Critical Points.*—We shall say that a continuous deformation D of a subset A of M admits a *displacement function* $\delta(e)$ on A , if whenever q precedes r on a trajectory of D and $qr > e > 0$, then $F(q) - F(r) > \delta(e)$, where $\delta(e)$ is a positive single-valued function of e . A continuous deformation of E which possesses a displacement function on each compact subset of E is termed an *F -deformation* of E . A point p will be termed *homotopically ordinary* if some neighborhood of p relative to $F \leq F(p)$ admits an F -deformation which displaces p . A point p which is not homotopically ordinary is termed *homotopically critical*.

The function F will be said to be *upper-reducible* at p if corresponding to each constant $c > F(p)$ some neighborhood of p relative to $F \leq c$ admits an F -deformation onto a set d-below c . A function F which is lower semi-continuous is not necessarily upper-reducible, and conversely. We have the following principal theorem.

THEOREM 3.1. *If F is upper-reducible at each point, each cap limit is assumed by F in at least one homotopic critical point.*

If then the hypothesis of F -accessibility is satisfied and F is upper-reducible each cycle limit $s(u)$ is assumed by F at some homotopic critical point. This should be contrasted with the following theorem: When the space M is compact and F is lower semi-continuous, the absolute minimum of F is assumed at some critical point. As ever $F \geq 0$.

By the *complete critical set* ω at the level c is meant the set of all homotopic critical points at which $F = c$. Any subset σ of ω which is closed in ω and at a positive distance from $\omega - \sigma$ will be termed a *critical set*. A k -cap u with cap limit c will be said to be *associated* with σ if u is c -homologous to a k -cap on an arbitrarily small neighborhood of σ . A maximal group of k -caps associated with σ will be called the *k th type group* of σ . Any two k th type groups of σ are rank isomorphic (with the ranks the cap limits). The dimension of a k th type group of σ is termed the *k th type number* of σ . A k th type group of ω can be obtained as a direct sum of the k th type groups

of any finite set of disjunct critical sets summing to ω . We have the following theorem:

THEOREM 3.2. *If M is F -accessible and F is upper-reducible on $F < 1$, the sum of the k th type numbers of the respective critical sets on $F < 1$ is at least the smaller of the two cardinal numbers, alef-null and the k th connectivity of $F < 1$.*

In the special case where F is locally a function of class C^n of n coördinates, and p is a critical point at which the Hessian of F is not zero, the j th type number of p equals the Kronecker δ_k^j , where k is the number of negative characteristic roots of the Hessian of F at p .

4. *Variational Theory.*—We apply the preceding theory to the problem of finding extremals joining two points a and b of a connected space Σ with a symmetric metric pq . The space of all sensed curves joining a to b on Σ with a Fréchet distance between curves will be denoted by $\Omega(a, b)$. The space $\Omega(a, b)$ here replaces M . We begin by showing that the k th homology group of $\Omega(a, b)$ is isomorphic with that of $\Omega(a', b')$, provided Σ is arcwise connected. To define F on Ω we suppose that we have a second metric $[pq]$ defined for p and q on Σ . We do not assume that $[pq] = [qp]$. Otherwise $[pq]$ shall satisfy the usual axioms. We assume that $[pq]$ is continuous in p and q in terms of the first metric pq . The function $J(\lambda)$ shall be the length of the curve λ of $\Omega(a, b)$ defined in the usual way in terms of the second metric $[pq]$. We set

$$F(\lambda) = \frac{J(\lambda)}{1 + J(\lambda)}$$

with $F(\lambda) = 1$ when $J(\lambda)$ is infinite.

We assume that Σ is *finitely J-compact* in that for each fixed point p of Σ and finite constant c , the subset $[pq] \leq c$ of Σ is compact. It follows that the hypothesis of F -accessibility is satisfied on $\Omega(a, b)$. A simple sensed curve λ joining two points p and q of Σ will be termed a *right arc* if a point r lies on λ when and only when $[pq] = [pr] + [rq]$. We assume that Σ is *locally J-convex* in the following sense. With each point p of Σ there shall be associated a positive number $\rho(p)$ continuous in p and such that when $q \neq p$ and $[pq] \leq \rho(p)$, p can be joined to q on Σ by a right arc every subarc of which is a right arc. It follows that F is upper-reducible on the subspace $F < 1$. This is sufficient for our purposes. A curve h will be called a *metric extremal* provided every closed subarc of h whose J -length is sufficiently small is a right arc. Regarded as a curve each homotopic critical point of F will be called a *homotopic extremal*. We have the following fundamental theorem.

THEOREM 4.1. *Each homotopic extremal of $\Omega(a, b)$ is a metric extremal.*

We also show that $\Omega(a, b)$ is locally F -connected, that all cycle limits are less than 1, and that the subsets $F \leq c < 1$ are compact. The preceding

theory is readily applied to the calculus of variations with the usual positive, and positive regular integrand in parametric form.

¹ Morse, "Functional Topology and Abstract Variational Theory," *Annals of Mathematics*, 38, 386-449 (1937). We refer to this paper by the letter M. Complete references are given in this paper and in the fascicle to appear later.

² The following book will appear shortly: Seifert und Threlfall, *Variationsrechnung im Grossen. Theorie von Marston Morse*. Teubner, Berlin. This book is highly recommended. The authors begin with two axioms similar to our accessibility hypothesis, but referring to singular cycles. These axioms are satisfied when the critical values cluster at most at infinity and when the critical points are isolated. In this way the most important cases are treated in the simplest way. To obtain greater generality Vietoris cycles seem to be useful. In fact the present author has shown in 3 (following) that the accessibility hypothesis is not in general satisfied when ordinary cycles are used, even when f is of class C^n on regular analytic manifolds and when the critical values are finite in number.

³ Morse, "Sur le calcul des variations," *Bull. Société Mathématique de France* (1938).

ON CRITERIA CONCERNING SINGULAR INTEGERS IN CYCLOTOMIC FIELDS

BY H. S. VANDIVER

DEPARTMENT OF PURE MATHEMATICS, UNIVERSITY OF TEXAS

Communicated June 24, 1938

As elsewhere¹ a singular integer is defined as an integer α in the field $k(\zeta)$; $\zeta = e^{2i\pi/l}$, l an odd prime, such that $\alpha = a'$ where a is an ideal in $k(\zeta)$ which is not principal. Necessary conditions that an integer in $k(\zeta)$ be singular were given by Takagi,² and when the field $k(\zeta)$ is properly irregular, that is to say, when the second factor of its class number is prime to l ; a necessary and sufficient condition was given by the writer.¹ Here we shall give some other necessary conditions for singular integers in any irregular cyclotomic field. Based on a result of Kummer's the writer³ obtained the relation

$$\prod_{s=1}^{k-1} \prod_{r=1}^{[sl/k]} b(\zeta^{sr}) \sim 1, \quad (1)$$

that is, the ideal on the left is principal, where k is an integer $1 < k < l$; $sr_1 \equiv 1 \pmod{l}$; $[s]$ is greatest integer in s , and b is any integer in $k(\zeta)$ and $b(\zeta^r)$ is obtained from $b(\zeta)$ by the substitution (ζ/ζ^r) ; and it follows if we assume that (Vandiver³) α is singular and semi-primary, then

$$\prod_{s=1}^{k-1} \prod_{r=1}^{[sl/k]} \alpha(\zeta^{sr}) = \omega^l$$

where ω is an integer in $k(\zeta)$. As it stands this is a necessary condition that α be singular, but we shall show that this can be transformed into criteria which do not involve k . The method is an extension, and modification, of one employed previously by the writer⁴ in connection with units in certain cyclic fields. We shall find it convenient to employ power characters in $k(\zeta)$. Set

$$\left(\frac{\theta}{\mathfrak{p}}\right) = \zeta^{I(\theta)}$$

and

$$\theta^{\frac{N(\mathfrak{p})-1}{l}} \equiv \left(\frac{\theta}{\mathfrak{p}}\right) \pmod{\mathfrak{p}}$$

where \mathfrak{p} is any ideal in $k(\zeta)$ prime to (θ) and (l) with θ an integer in $k(\zeta)$. Also put

$$D_s = \sum_{d=1}^{l-1} d^s I(\alpha(\zeta^d))$$

and consider the sum

$$\sum_{s=0}^{l-2} \sum_{d=1}^{l-1} d_1^{l-1-s} d^s I(\alpha(\zeta^d))$$

where d_1 is one of the integers in the set $1, 2, \dots, l-1$. This may be put in the form

$$(l-1) I(\alpha^{d_1}) + \sum_{d_1 \neq d} d_1 \frac{d_1^{l-1} - d^{l-1}}{d_1 - d} I(\alpha(\zeta^d))$$

whence

$$-I(\alpha(\zeta^d)) \equiv D_0 + d^{l-2} D_1 + d^{l-3} D_2 + \dots + d D_{l-2},$$

modulo l . Applying this to (1) after setting ζ^m for ζ we have, if $\mu = (l-1)/2$,

$$\begin{aligned} \mu(k-1)D_0 m^{l-1} + \sum_r (mr_1)^{l-2} D_1 \\ + \sum_r (mr_1)^{l-3} D_2 + \dots + \sum_r (mr_1) D_{l-2} \equiv 0 \pmod{l}. \end{aligned}$$

Now set $m = 1, 2, \dots, l-1$ in turn we obtain $(l-1)$ congruences and we obtain by elimination

$$\begin{aligned} D_0 \equiv \sum r_1^{l-s} D_{s-1} \equiv 0 \pmod{l}, \\ s = 2, 3, \dots, l-1. \end{aligned} \tag{2}$$

Using the known relation

$$\frac{(1-k^i)b_i}{k^{i-1}i} \equiv \sum r_1^{l-i} \pmod{l},$$

$$b_1 = -1/2, b_{2a+1} = 0, a > 0; \quad b_{2a} = (-1)^{a-1} B_a,$$

the B 's being the numbers of Bernoulli, $B_1 = 1/6$, $B_2 = 1/30$, etc., and letting k be a primitive root of l then (2) gives

$$D_0 \equiv b_{s+1} D_s \equiv 0 \pmod{l}, \quad (3)$$

$s = 1, 2, \dots, l-2$. Suppose that $b_{2a} \not\equiv 0 \pmod{l}$ then (3) gives

$$D_{2a-1} \equiv 0 \pmod{l},$$

$$\left(\frac{\prod_{d=1}^{l-1} \alpha(\zeta^d)^{d^{2a-1}}}{p} \right) = 1,$$

for any p prime to l and $N(\alpha)$. Hence by a known result we have the
THEOREM: *If $\alpha(\zeta)$ is a singular integer in the field $k(\zeta)$ and is also semi-primary; $\zeta = e^{2i\pi/l}$, l an odd prime and $B_a \not\equiv 0 \pmod{l}$, $a < (l-1)/2$, then*

$$A_{2a-1} = \prod_{d=1}^{l-1} (\alpha(\zeta^d))^{d^{2a-1}} = \sigma^l \quad (4)$$

where σ is an integer in $k(\zeta)$.

If in (4) we obtain an identity in e^v by adding a suitable multiple of $(l^v - 1)/(l^v - 1)$ and differentiate $(l-2a)$ times and set $v = 0$ we obtain

$$\left[\frac{d^{l-2a} \log \alpha(l^v)}{dv^{l-2a}} \right]_{v=0} \equiv 0 \pmod{l}$$

which gives a known criterion that $\alpha(\zeta)$ is singular.

We also note that

$$A_{2a-1} = \prod_{c=0}^{l-2} (\alpha(\zeta^{r^c}))^{r^{c(2a-1)}} \delta$$

where now r is a primitive root of l and also

$$A_{2a-1}(\zeta^r)^{r^{2a-1}} = A_{2a-1}(\zeta) \gamma^l$$

where δ and γ are numbers in $k(\zeta)$.

Additional relations of this type hold if we restrict ourselves to properly irregular cyclotomic fields. Here for any singular integer in $k(\zeta)$, say, $\omega(\zeta)$, we have

$$\omega(\zeta) \omega(\zeta^{-1}) = \epsilon \tau^l$$

where ϵ is a unit and τ an integer in $k(\zeta)$. Treating this relation in a similar manner to that employed in connection with (1) we obtain

$$\prod_{d=1}^{l-1} (\omega(\zeta^d))^{d^{2a}} = \beta^l \delta$$

for each a in the set $1, 2, \dots, (l-3)/2$, such that δ is a unit in $k(\zeta)$ with $\delta(\zeta^r)^{r^{1a}} = \delta(\zeta)\gamma^l$, and $B_a \equiv 0 \pmod{l}$.

¹ Vandiver, these PROCEEDINGS, 15, 203 (1929).

² *Jour. für die Reine und Angewandte Mathematik*, 157, 230-238 (1927).

³ *Ann. Math.* (2), 21, 78 (1919).

⁴ *Trans. Amer. Math. Soc.*, 29, 156 (1927).

GROUPS OF DEGREE n INVOLVING ONLY SUBSTITUTIONS OF LOWER DEGREES

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated July 7, 1938

Every transitive group of degree n involves at least $n-1$ substitutions of this degree but an intransitive group of degree n does not necessarily involve any substitution whose degree is as large as n . Such intransitive groups exist only when $n > 4$ and there is obviously only one such group of degree 5, viz., the group formed by a 3, 1 isomorphism between the symmetric group of degree 3 and the group of degree 2. Whenever n is odd and exceeds 3 it is clearly possible to construct similarly a group of degree n which does not involve any substitution whose degree is as large as n by dimidiating the dihedral group of degree $n-2$ and the group of degree 2. Exactly half of the substitutions of this group are of degree $n-1$ while the remaining substitutions thereof appear in the cyclic subgroup of degree $n-2$ and are either of degree zero or of degree $n-2$.

From the preceding paragraph it results that when n is odd and exceeds 3 it is always possible to construct at least one group of degree n which does not involve any substitution of this degree and has one transitive constituent of degree $n-2$. We proceed to prove that such a group cannot be constructed when n is an even number. If this were possible the transitive constituent of degree $n-2$ of such a group would involve a subgroup of index 2 containing all its substitutions of degree $n-2$ since none of its other substitutions would be of as large a degree as $n-2$. Hence all of these other substitutions would be of degree $n-3$ since this is the average number of letters in the substitutions of this constituent. As $n-3$ is supposed to be odd each of these substitutions of degree $n-3$ involves an odd cycle and an odd power of such a substitution would involve less than $n-3$ letters but would appear among the given substitutions of degree $n-3$. As this is impossible it results that a necessary and sufficient condition

that there exists a group of degree $n > 3$ which involves no substitution of degree n but has a transitive constituent of degree $n - 2$ is that n is odd.

Suppose that n is the sum of two distinct prime numbers p, q , $p > q$, and that a group has two transitive constituents of degree p and q , respectively. If $p - 1$ is divisible by q it is clearly possible to construct a group of degree n which involves no substitution of degree n by establishing a $p, 1$ isomorphism between a subgroup of the metacyclic group of order $p(p - 1)$ and the group of degree q . On the other hand, when $p - 1$ is not divisible by q it is impossible to construct a group of degree n which does not involve a substitution of degree n since every invariant subgroup of a transitive group of degree p involves all of its substitutions of order p and its quotient group under the entire group is cyclic and has an order which divides $p - 1$.¹ For the same reason when $n = 2p$, where p is an odd prime number, it is impossible to construct a group of degree n which does not contain a substitution of degree n . Hence there results the following theorem: *When n is the sum of two prime numbers it is impossible to construct a group of degree n which has these numbers for the degrees of its transitive constituents and involves no substitution of degree n unless the larger of them is congruent to unity with respect to the smaller.*

If at least one of the constituent groups of a group involves no substitution whose degree is equal to the degree of this constituent then the entire group cannot involve a substitution whose degree is equal to the degree of the group. In particular, if at least one of the factor groups of a direct product involves no substitution whose degree is equal to the degree of this factor group then the entire group will obviously have the same property. Since the degree of such a factor group need not exceed 5 it results that whenever $n > 6$ there is at least one direct product of degree n which has the property that it contains no substitution whose degree is equal to the degree of this direct product. It is easy to verify that no such group exists whenever $n < 7$ and that the only group of degree 6 which contains no substitution of this degree is composed of the positive substitutions of the group of order 8 and degree 6 which has three transitive constituents.

In what precedes, two substitution groups of a given degree are said to be identical whenever they are conjugate under the symmetric group of this degree. It is often desirable to consider two such groups as distinct whenever one of them contains at least one substitution which does not appear in the other. If this is done there are 10 groups of degree 5 which have the property that none of them contains a substitution of this degree since such a group is transformed into itself by 12 substitutions on these 5 letters. Similarly there are 15 groups of degree 6 which separately do not involve a substitution of this degree since each of them is transformed into itself by 48 substitutions on these 6 letters. In what follows we shall employ the latter of these two methods of enumerating substitution groups unless the

contrary is stated. There are therefore 210 groups on 7 letters, each being of degree 5, which separately have the property that none of them involves a substitution which is equal to the degree of the group, and there are 105 groups on 7 letters, each being of degree 6, which separately have the same property.

It was noted above that whenever $n > 6$ there is always at least one direct product of degree n which does not contain a substitution of this degree. We proceed to prove that there is then also at least one group of this degree involving no substitution of this degree but in which every constituent group contains at least one substitution whose degree is equal to the degree of this constituent. When n is odd such a group can obviously be constructed by establishing a dimidiation between the dihedral group of degree $n - 2$ and the group of degree 2 on the remaining letters. When n is even the dihedral group of degree $n - 4$ involves $(n - 4)/2$ substitutions of degree $n - 6$ and the same number of substitutions of degree $n - 4$ besides the cyclic subgroup of this order. Hence we can establish an isomorphism between this dihedral group and the intransitive group of order 4 on the remaining letters so that no substitution has a degree which exceeds $n - 2$ and all the substitutions are of this degree except those in the cyclic subgroup of order $(n - 4)/2$. As every constituent group of this group involves at least one substitution whose degree is equal to the degree of this constituent there results the following theorem: *There is at least one group of every degree $n > 6$ which has the property that it does not involve any substitution of degree n and that each of its constituent groups involves at least one substitution whose degree is equal to the degree of this constituent.*

To obtain an expression for the number of the groups in each of the two categories noted in the preceding paragraph it may be observed that when n is odd the given cyclic group of degree $n - 2$ can be chosen in $n(n - 1) \dots 3/(n - 2)$ ways and one such group corresponds to each of these cyclic subgroups. When n is even the given cyclic group of degree $n - 4$ can be selected in $n(n - 1) \dots 5/(n - 4)$ ways but for each of these cyclic subgroups there are three of the required groups. Hence the number of the possible groups which satisfy the given condition in this case is $3n(n - 1) \dots 5/(n - 4)$. When n is odd the given number of these groups exceeds $(n - 1)!/2$. This is of interest in connection with the question of the relative number of substitutions and subgroups in the symmetric group of degree n .

If a group of degree n is constructed by establishing a simple isomorphism between symmetric groups of degree m it necessarily involves substitutions of degree n . To prove this theorem it may first be noted that when $m \neq 6$ then transpositions of these various symmetric groups correspond to each other in this simple isomorphism in view of the fact that when $m > 6$ a transposition is transformed into itself by more substitu-

tions of the symmetric group than any other substitution of order 2 contained therein. Hence these transpositions correspond to each other in such a simple isomorphism except possibly when $m = 6$. In this particular case the positive substitutions of order 4 of such a symmetric group correspond to each other and hence the resulting group contains substitutions of degree n and does not require further consideration here. In all other cases a transposition and a cyclic substitution of degree $m - 1$ which involves one and only one letter of this transposition generate the symmetric group of degree m . The latter therefore corresponds also to such a substitution. As the product of these two substitutions is cyclic and of degree m it results that every group of degree n which is formed by a simple isomorphism between symmetric groups involves at least one substitution of degree n .

Suppose that a group has the property that all its operators appear in two sets of conjugate subgroups which are separately neither invariant nor contain any invariant subgroup of the entire group besides the identity. Such a group can be represented as a transitive substitution group with respect to each of these sets of conjugate subgroups and these two transitive representations of the group can be placed into a simple isomorphism in such a way that the resulting group of degree n contains no substitution of degree n . As an illustration of such a group we cite the symmetric group of degree 4 which may be represented as a transitive group of degree 4 with respect to its subgroups of order 6 and as a transitive group of degree 6 with respect to its cyclic subgroups of order 4. The two groups thus obtained can be placed either into a simple isomorphism or into a 4, 4 correspondence so as to obtain a group of degree 10 which involves no substitution whose degree exceeds 9. In the alternating group of degree 5 all the substitutions appear either in the conjugate subgroups of order 12 or in the conjugate subgroups of orders 5 or 10. Simple isomorphisms between these groups can therefore be established in more than one way so as to obtain groups containing no substitution, whose degree is equal to the degree of the group.

¹ G. A. Miller, *Bull. Amer. Math. Soc.*, 4, 141 (1898).

ON THE DERIVATIVES OF FUNCTIONS ANALYTIC IN THE UNIT CIRCLE

BY J. L. WALSH AND W. SEIDEL

DEPARTMENTS OF MATHEMATICS, HARVARD UNIVERSITY AND UNIVERSITY OF ROCHESTER

Communicated July 5, 1938

Various results concerning the order of growth of the first and higher derivatives of univalent and of bounded functions analytic in the unit circle are known. Among these we mention Koebe's distortion theorem (Verzerrungssatz) in the univalent case and Schwarz's Lemma and the results of O. Szász¹ in the bounded case. A consequence of these results for a function $f(z)$ analytic in $|z| < 1$ is $|f'(z)| = O\left(\frac{1}{(1 - |z|)^2}\right)$ in the case that $f(z)$ is univalent and $|f^{(n)}(z)| = O\left(\frac{1}{(1 - |z|)^n}\right)$ in the case that $f(z)$ is bounded. These relations, however, give no information as to the conditions under which $|f^{(n)}(z_k)|(1 - |z_k|)^n$ tends to zero for a given sequence of points $\{z_k\}$ in the unit circle. In the univalent case an answer to this question is contained in the following result due to J. E. Littlewood and A. J. Macintyre:² *Let $f(z)$ be analytic and univalent in $|z| < 1$ and let it omit there the value ω . Then in $|z| < 1$ the following inequality is satisfied:*

$$|f'(z)|(1 - |z|^2) \leq 4 |\omega - f(z)|.$$

The purpose of the present note is to summarize the principal results recently obtained by the authors in the study of the relation

$$|f^{(n)}(z_k)|(1 - |z_k|)^n \rightarrow 0 \text{ as } k \rightarrow \infty, \text{ where } |z_k| < 1,$$

in the case that the analytic function $f(z)$ is univalent, or is bounded, or omits two values in $|z| < 1$. The method involves primarily the systematic and detailed study of the function $\varphi_k(\zeta) = f\left(\frac{z_k + \zeta}{1 + \bar{z}_k\zeta}\right)$. The detailed exposition will be published at a later date.

Let $w = f(z)$ be analytic in $|z| < 1$ and let R denote the Riemann configuration over the w -plane onto which this function maps the region $|z| < 1$. Let w_0 be an arbitrary point of R . Then the radius of the largest smooth circle (boundary not included) with center at w_0 and wholly contained in R is called the *radius of univalence of R at w_0* and will be denoted by $D_1(w_0)$. The following theorem can be easily proved:

Let $f(z)$ be regular and univalent in $|z| < 1$, z_0 any point of $|z| < 1$, and $w_0 = f(z_0)$. Then

$$D_1(w_0) \leq |f'(z_0)|(1 - |z_0|^2) \leq 4D_1(w_0). \quad (1)$$

Each of these inequalities is sharp and the second one is another form of the Littlewood-Macintyre inequality. An immediate consequence of this theorem is the corollary:

Let $f(z)$ be regular and univalent in $|z| < 1$, z_n any sequence of points in $|z| < 1$, and $w_n = f(z_n)$. Then a necessary and sufficient condition that

$$\lim_{n \rightarrow \infty} |f'(z_n)|(1 - |z_n|) = 0 \quad (2)$$

is that

$$\lim_{n \rightarrow \infty} D_1(w_n) = 0, \quad (3)$$

and a necessary and sufficient condition that $|f'(z_n)|(1 - |z_n|)$ be bounded is that $D_1(w_n)$ be bounded.

From this theorem it follows for a univalent and bounded function $f(z)$ that $|f'(z)| = o\left(\frac{1}{1 - |z|}\right)$ uniformly in the circle $|z| < 1$ as $|z| \rightarrow 1$. In this connection the following theorem is appropriate:

Let $f(z)$ be regular and univalent in the circle $|z| < 1$. Then

$$\lim_{z \rightarrow e^{i\alpha}} |f'(z)|(1 - |z|)^{1/2} = 0$$

for all points $e^{i\alpha}$ of the circumference $|z| = 1$ with the exception of at most a set of Lebesgue measure zero, where z in the above limit is taken in any angle less than π with vertex in $e^{i\alpha}$ and bisected by the corresponding radius.

For univalent functions the second part of inequality (1) admits of extensions to all higher derivatives, for instance

$$|f^{(n)}(z_0)|(1 - |z_0|^2)^n \leq 4 \cdot e \cdot n! (|z_0| + n)(1 + |z_0|)^{n-2} D_1(w_0).$$

For $n = 2$ or 3 the factor e in the right side may be omitted; the resulting inequalities are sharp. Obviously, $D_1(w_k) \rightarrow 0$, $w_k = f(z_k)$, implies

$$|f^{(n)}(z_k)|(1 - |z_k|)^n \rightarrow 0. \quad (4)$$

Examples can be constructed to show that the limit in (2) and in (4) can be approached arbitrarily slowly even if $f(z)$ is univalent and continuous in $|z| \leq 1$.

The situation is entirely analogous for bounded functions:

Let $f(z)$ be regular and bounded in $|z| < 1$:

$$|f(z)| \leq M,$$

let $\{z_n\}$ be any sequence of points in $|z| < 1$, and let $w_n = f(z_n)$. Then a necessary and sufficient condition for (2) is (3).

Indeed, for any z_0 ($|z_0| < 1$) we have

$$D_1(w_0) \leq |f'(z_0)|(1 - |z_0|^2) \leq \sqrt{8MD_1(w_0)}, \quad (5)$$

where $w_0 = f(z_0)$.

The situation for the higher derivatives of bounded functions is somewhat more complicated than in the univalent case. We need the following definitions:

Let R be a Riemann surface (configuration) over the w -plane, let w_0 be any point of R not a branch point of order greater than $p - 1$. Suppose that C_p is a simply connected region on R which contains w_0 in its interior, lies over the circle $|w - w_0| < \rho$, and covers this circle precisely p times. We call such a region a p -sheeted circle of center w_0 and radius ρ ; the value $\rho = \infty$ is not excluded. The radius of p -valence $D_p(w_0)$ of a Riemann surface R at a point w_0 belonging to R is defined as follows:

- (a) For $p = 1$, $D_p(w_0) = D_1(w_0)$.
- (b) If w_0 is a branch point of order greater than $p - 1$ ($p > 1$), then $D_p(w_0) = 0$.
- (c) For any other point w_0 , the number $D_p(w_0)$ is the radius of the largest p -sheeted circle with center w_0 contained in R if such a circle exists, and is otherwise $D_{p-1}(w_0)$.

With this definition we prove:

Let $\{f_n(z)\}$ be a sequence of functions analytic in the unit circle $|z| < 1$ and converging uniformly in every closed subregion of $|z| < 1$ to an analytic function $f(z)$. Let z_0 be any point in the circle $|z| < 1$ and set $w_n = f_n(z_0)$, $w_0 = f(z_0)$. Let $D_p(w_n)$ and $D_p(w_0)$ pertain to the images of $|z| < 1$ by the maps $w = f_n(z)$ and $w = f(z)$, respectively. Then

$$\lim_{n \rightarrow \infty} D_p(w_n) = D_p(w_0).$$

As an analogue of the inequalities (5) we obtain:

Let $f(z)$ be regular and bounded in $|z| < 1$: $|f(z)| \leq M$, let z_0 be any point of $|z| < 1$ and $w_0 = f(z_0)$. Let p be a positive integer. Then there exist two positive constants λ_p depending only on p and Λ_p depending on p and M such that we have

$$\lambda_p \cdot D_p(w_0) \leq \sum_{k=1}^p \frac{1}{k!} \left| \sum_{\nu=0}^{k-1} (-1)^{\nu} \nu! \binom{k}{\nu} \binom{k-1}{\nu-1} \bar{z}_0^{\nu} (1 - |z_0|^2)^{k-\nu} \right| \cdot |f^{(k-\nu)}(z_0)| \leq \Lambda_p [D_p(w_0)]^{2^{-p}}. \quad (6)$$

Consequently, for any sequence $\{z_n\}$ ($|z_n| < 1$), a necessary and sufficient condition for

$$\lim_{n \rightarrow \infty} f^{(k)}(z_n) (1 - |z_n|)^k = 0 \quad (k = 1, 2, \dots, p)$$

with $w_n = f(z_n)$, is that

$$\lim_{n \rightarrow \infty} D_p(w_n) = 0.$$

The last part of this theorem may also be inferred from the preceding theorem. Constants λ_p and Λ_p for which (6) holds can be explicitly determined.

Schottky's theorem enables us to extend the above results for bounded functions to the case of functions $f(z)$ omitting two values provided the sequence $w_n = f(z_n)$ is bounded. The case $|w_n| \rightarrow \infty$ can be treated by other methods.

¹ O. Szász, *Math. Zeitschrift*, 8, 303-309 (1920).

² J. E. Littlewood, *Proc. London Math. Soc.*, 23, 507 (1924); A. J. Macintyre, *Jour. London Math. Soc.*, 11, 7-11 (1936).

DIFFERENTIAL CALCULUS IN LINEAR TOPOLOGICAL SPACES¹

BY A. D. MICHAL

DEPARTMENT OF MATHEMATICS, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated July 9, 1938

1. *Introduction.*—The most valuable definitions of differentials of functions in the classical differential calculi of finite as well as of infinite dimensional spaces are those that give the differential as a "first order approximation" to the difference. In this paper we give a definition of such a differential for functions whose arguments are in a linear topological space T_1 and whose values are in a linear topological space T_2 , not necessarily the same² as T_1 . Some of the fundamental properties of this differential are given as well as the properties of other related topological differentials.

We wish to emphasize here the fact that the spaces T_1 and T_2 are not necessarily metric—not even metrizable—and that the differential calculus in linear topological spaces has important applications to general differential geometry, general dynamics and general continuous group theory.

2. *Topological M-Differential.*—By a linear topological space we shall mean an abstract linear space with a Hausdorff topology in which the functions $x + y$ and αx are respectively continuous functions of both variables.

Let T_1 and T_2 be any two linear topological spaces. A function $l(x)$ on T_1 to T_2 is termed *linear* if it is additive and continuous—hence homogeneous of degree one.

DEFINITION OF M-DIFFERENTIAL.³ Let $f(x)$ be a function with values in T_2 and defined on a Hausdorff neighborhood S_x of $x_0 \in T_1$. The function $f(x)$ will be said to be M-differentiable at $x = x_0$ and $f(x_0; \delta x)$ will be called an M-differential of $f(x)$ at $x = x_0$ with increment δx if

(1) there exists a linear function $f(x_0; \delta x)$ of δx with arguments in T_1 and values in T_2

(2) there exists a function $\epsilon(x_0, x_1, x_2)$ with arguments in T_1 and values in T_2 such that

$$(a) \quad \epsilon(x_0, 0, x) = 0 \text{ for all } x \in T_1$$

$$(b) \quad \epsilon(x_0, x_1, \lambda x_2) = \lambda \epsilon(x_0, x_1, x_2)$$

for all $\lambda > 0$, for all x_1 in some Hausdorff neighborhood of $0 \in T_1$, and for all $x_2 \in T_1$.

$$(c) \quad \epsilon(x_0, x_1, x_2)$$

is continuous in (x_1, x_2) at $x_1 = 0, x_2 = x_2$ for all $x_2 \in T_1$.

(3) there exists some Hausdorff neighborhood S_0' of $0 \in T_1$ such that for all $\delta x \in S_0'$

$$f(x_0 + \delta x) - f(x_0) - f(x_0; \delta x) = \epsilon(x_0, \delta x, \delta x).$$

THEOREM 1. If an M -differential of $f(x)$ at $x = x_0$ exists, then it is unique and $f(x)$ is continuous at $x = x_0$.

THEOREM 2. If $f_1(x)$ and $f_2(x)$ are M -differentiable at $x = x_0$ then $f_3(x) = \alpha f_1(x) + \beta f_2(x)$ is M -differentiable at $x = x_0$ and

$$f_3(x_0; \delta x) = \alpha f_1(x_0; \delta x) + \beta f_2(x_0; \delta x).$$

THEOREM 3. Let T_3 be a third linear topological space. If $f(x)$ on $S_{x_0} \subset T_1$ to T_2 is M -differentiable at $x = x_0$ and if $\phi(y)$ on $f(S_{x_0})$ to T_3 is M -differentiable at $y_0 = f(x_0)$, then $\psi(x) = \phi(f(x))$ is M -differentiable at $x = x_0$ and

$$\psi(x_0; \delta x) = \phi(f(x_0); f(x_0; \delta x)).$$

3. OTHER DIFFERENTIALS AND THEIR RELATION TO THE M -DIFFERENTIAL. **DEFINITION OF G -DIFFERENTIAL.** Let $f(x)$ be a function defined on a Hausdorff neighborhood S_{x_0} of $x_0 \in T_1$ and with values in T_2 . We shall say that $f(x)$ is G -differentiable at $x = x_0$ and $f(x_0; \delta x)$ is its G -differential at $x = x_0$ with increment δx if for any chosen $\delta x \in T_1$:

Given any Hausdorff neighborhood V_0 of $0 \in T_2$ there exists a $\delta > 0$ such that⁴

$$\frac{f(x_0 + \lambda \delta x) - f(x_0)}{\lambda} \in f(x_0; \delta x) + V_0$$

for each λ satisfying $0 < |\lambda| < \delta$.

THEOREM 4. If $f(x)$ is M -differentiable at $x = x_0$, then $f(x)$ is G -differentiable at $x = x_0$ and the two differentials are equal.

DEFINITION OF H M -DIFFERENTIAL.⁵ Let $f(x)$ be a function with values in T_2 and defined on a Hausdorff neighborhood S_{x_0} of $x_0 \in T_1$, and let $x(\lambda)$ be any chosen function of a real variable λ with values in S_{x_0} and possessing a derivative $\frac{dx(\lambda)}{d\lambda}$ at any chosen $\lambda = \lambda_0$. Write $x_0 = x(\lambda_0)$. The function $f(x)$

will be said to be H M -differentiable at $x = x_0$ with $f(x_0; \delta x)$ as its H M -dif-

ferential at $x = x_0$ if there exists a linear function $f(x_0; \delta x)$ of δx having arguments in T_1 and values in T_2 such that for every admissible $x(\lambda)$:

$$(1) \quad \frac{d}{d\lambda} f(x(\lambda)) \text{ exists at } \lambda = \lambda_0$$

$$(2) \quad \frac{d}{d\lambda} f(x(\lambda)) = f\left(x_0; \frac{dx(\lambda)}{d\lambda}\right) \text{ for } \lambda = \lambda_0.$$

THEOREM 5. If $f(x)$ is M -differentiable at $x = x_0$, then $f(x)$ is $H M$ -differentiable at $x = x_0$ and the two differentials are equal.

THEOREM 6. If the linear topological spaces T_1 and T_2 are complete linear normed spaces (Banach spaces) and if $f(x)$ is Fréchet differentiable⁶ at $x = x_0$, then $f(x)$ is M -differentiable at $x = x_0$ and the two differentials are equal.

THEOREM 7. If the linear topological spaces T_1 and T_2 are finite dimensional arithmetic spaces and if $f(x)$ is differentiable at $x = x_0$ in the Stolz-Young-Fréchet sense, then $f(x)$ is M -differentiable at $x = x_0$ and the differentials are equal. Conversely if $f(x)$ is M -differentiable at $x = x_0$, then it is differentiable in the Stolz-Young-Fréchet sense.

¹ Presented to the American Mathematical Society, April 9, 1938.

² In case T_1 is a special linear topological space and T_2 is the same space as T_1 , a certain topological differential was defined by Michal and Paxson. It is still an open question whether the differentiability theorem on the composition of functions is valid for the Michal-Paxson differential. See Michal, A. D., and Paxson, E. W.: (1) "La Différentielle dans les Espaces Linéaires Abstraits avec une Topologie," *Comptes Rendus, Paris*, 202, 1741-1743 (1936); (2) "The Differential in Abstract Linear Spaces with a Topology," *Comptes Rendus de la Soc. de Sc. de Varsovie*, XXIX, 106-121 (1936).

³ Another interesting type of differential can be defined by merely changing the equality relation in condition (3) of the definition for an M -differential into a set inclusion relation.

⁴ By $f(x_0, \delta x) + V_0$ we mean the set of all elements $f(x_0, \delta x) + y$ as y ranges over the set V_0 .

⁵ A modified $H M$ -differential is obtained if λ_0 is always taken to be $\lambda_0 = 0$. This modified $H M$ -differential is itself the abstraction of a differential for a function space studied recently by Fréchet. See page 244 of Fréchet, M., "Sur la Notion de Différentielle," *Journal de Math. Pures et Appl.*, 16, 233-250 (1937).

⁶ Fréchet, M., "La Notion de Différentielle dans l'Analyse Générale," *Annales Ecole Norm.*, XLII, 293-323 (1925).

MINIMAL SURFACES OF HIGHER TOPOLOGICAL STRUCTURE

BY JESSE DOUGLAS

BROOKLYN, N. Y.

Communicated June 30, 1938

1. About two years ago, the author published a solution of the Plateau problem in the following general topological form.

Given:

(1) k contours Γ in the form of Jordan curves in n -dimensional euclidean space, each of assigned form, position and sense of description;

(2) a prescribed genus h or topological characteristic r ;¹

(3) either character of orientability, i.e., two-sided or one-sided;

to determine a minimal surface M corresponding to these data.

This problem had been formulated a number of years before by the author² and the important special cases $k = 1, r = 0$; $k = 2, r = 0$; $k = 1, r = 1$ (Möbius strip) solved during the intervening time.³ Indeed, the formulas of the paper on the two-contour case were explicitly developed in a form valid for the general case.⁴

The publication referred to at the beginning was in the form of two papers, the first of which stated the results, while the second gave proofs and other details.⁵

Subsequently, an alternative method of solution of the same problem was announced by R. Courant,⁶ who, the following year, published the details for the case of genus zero.⁷ As far as the case of one contour and a simply-connected minimal surface is concerned, Courant's method was presented independently by L. Tonelli,⁸ who remarks that it coincides substantially with the one used by the author throughout his work on the Plateau problem.

2. Early next year, the *Annals of Mathematics* will publish a comprehensive paper by the author with the same title as the present note. This paper first reviews and elaborates the essential parts of the author's previous papers on the subject, and then provides various supplementary considerations, relating particularly to the case of genus $h > 0$.

The purpose of the note at hand is to present a summary of some of these more novel features of the detailed exposition contained in the forthcoming *Annals* paper.⁹

3. To typify immediately the distinction between the cases $h = 0$ and $h > 0$, we write down the respective formulas for the Green's function of the basic Riemann surface R , namely,¹⁰

$$h = 0: G(z, w) = -\Re \Omega(z; w, \bar{w}); \quad (1)$$

$$h > 0: G(z, w) = -\Re \{ \Omega(z; w, \bar{w}) - T_{\alpha\beta} [v_\alpha(z) - v_{\alpha'}(z)] \\ [v_\beta(w, \bar{w}) - v_{\beta'}(w, \bar{w})] \}. \quad (2)$$

Here, $\Omega(z; w, \bar{w})$ denotes the *normal integral of the third kind*¹¹ with logarithmic singularities at the conjugate or symmetric points w, \bar{w} . The other notation and general background will appear in the course of the explanations that follow.

The properties which define $G(z, w)$ uniquely are: (I) *uniform* on R , (II) *harmonic* on R , (III) logarithmic singularity at $z = w$, with principal term $-\log |z - w|$, (IV) $G(z, w) = 0$ when z is on the boundary of R .

In the case $h = 0$, formula (1) has all these properties; but when $h > 0$, the property of *uniformity* is lost, since what we shall call *alter-symmetric circuits*¹² then appear on R , with respect to which circuits G has, in general, non-vanishing periods. The purpose of the complementary terms in (2) is precisely to *neutralize these periods and restore uniformity*, while still preserving the other essential properties of Green's function.

4. As standard domain on which to represent the required minimal surface M , we employ a "semi Riemann surface" R . This is either of the two conjugate halves of a complete Riemann surface \mathfrak{R} which is supposed to be *symmetric*, i.e., to have an inverse conformal transformation T into itself which associates the points of \mathfrak{R} in pairs P, \bar{P} , called *conjugate* or *symmetric*. Thus $T^2 = 1$. \mathfrak{R} always belongs as Riemann surface to a class of birationally equivalent real algebraic curves $\mathfrak{A}: P(x, y) = 0$, and T may be interpreted as the interchange of conjugate complex points $(x, y), (\bar{x}, \bar{y})$ of \mathfrak{A} . Then the real branches C of \mathfrak{A} remain, point by point fixed under T . They are said to constitute the *curves of transition* of \mathfrak{R} and may separate that surface or not. According as the former or the latter is the case, \mathfrak{R} is said to be of the *first kind* or *second kind*. These two kinds of Riemann surface correspond, respectively, to two-sided or to one-sided minimal surfaces M, \bar{M} . For if we regard a pair of symmetric points P, \bar{P} as a single geometric element, then \mathfrak{R} becomes a manifold R , which is one-sided or two-sided according as it is or is not possible to pass continuously from P to \bar{P} without traversing the boundaries of R ; and these boundaries are constituted by the transition curves C of the original surface \mathfrak{R} .

In the case of separation of \mathfrak{R} by C , either half may be identified as R ; and R , previously termed a semi Riemann surface, may also be described simply as a Riemann surface, except that R has the boundaries C , while \mathfrak{R} is closed. We require R to have exactly the topological structure prescribed for M , i.e., genus h and k boundaries. The genus of the complete surface \mathfrak{R} , on the other hand, is

$$p = 2h + k - 1 = r + k - 1 \quad (3)$$

The case of one-sided, or non-orientable, minimal surfaces \bar{M} can be dealt with by means of a two-sided, or orientable, *covering surface*, in the form of a Riemann surface \mathfrak{R} of the second kind, which is in two-one corre-

spondence with \bar{M} . That is, each pair of symmetric points P, \bar{P} of \mathfrak{R} correspond to the same point Q of \bar{M} . We may say that symmetric elements of \mathfrak{R} about P, \bar{P} correspond to elements of \bar{M} about the point Q which are geometrically coincident but antipodal, i.e., belong to opposite senses of the normal to \bar{M} .

Sufficiently typical is the case of the Möbius strip, where the covering surface of \bar{M} may be arranged as an orientable doubly-connected minimal surface M with two boundaries, which coincide with the opposite senses of description $+\Gamma, -\Gamma$ of the given contour Γ .¹³

5. In (1) and (2), the normal integral of third kind can be represented explicitly in terms of Riemann's multiple θ -functions as follows:

$$\Omega(z; w, \bar{w}) = \log \frac{\theta(v(z, w); \tau)}{\theta(v(z, \bar{w}); \tau)}. \quad (4)$$

Here $v(z, w)$ denotes the system of normal abelian integrals of the first kind, from the lower limit w to the upper limit z :

$$v_j(z, w) = v_j(z) - v_j(w) \quad (j, k = 1, 2, \dots, p). \quad (5)$$

τ denotes the matrix of periods of v . The multiple θ -series is defined explicitly by¹⁴

$$\theta(u; \tau) = \sum \exp [2\pi i u_j (n_j + \tfrac{1}{2} \alpha_j) + \pi i \tau_{jk} (n_j + \tfrac{1}{2} \alpha_j) (n_k + \tfrac{1}{2} \alpha_k) + \pi i \beta_j (n_j + \tfrac{1}{2} \alpha_j)], \quad (6)$$

where the summation convention as to repeated indices is applied to j, k and Σ denotes a multiple summation as n_1, n_2, \dots, n_p range over all integers from $-\infty$ to $+\infty$. The *characteristic* $\tfrac{1}{2} \beta, \tfrac{1}{2} \alpha$, consisting of $2p$ half-integers, usually 0 or $1/2$, is supposed to be *odd*, i.e.,

$$\beta\alpha = \beta_1\alpha_1 + \beta_2\alpha_2 + \dots + \beta_p\alpha_p = \text{odd integer.}^{15} \quad (7)$$

6. The Riemann surface \mathfrak{R} of genus p has $2p$ independent circuits: p of the *first system* A_j , and p of the *second system* B_j . These can be arranged in such a way that the inverse conformal automorphism T of \mathfrak{R} relates the indices j in pairs j, j' so that we have

$$TA_j = -A_{j'}, \quad TB_j = B_{j'}. \quad (8)$$

We shall call such indices j, j' *symmetric* to each other, and shall term the index j and the corresponding circuits A_j, B_j *self-symmetric* or *alter-symmetric* according as $j = j'$ or $j \neq j'$.

The essential feature of the case $h = 0$ is that then all indices and circuits are *self-symmetric*.

If, on the contrary, $h > 0$, then there are h alter-symmetric indices, belonging to the circuits A_α, B_α about the h handles of the semi-surface R .

These have corresponding symmetric circuits $A_{\alpha'}$, $B_{\alpha'}$ on the conjugate semi-surface R' .

As notation, we use $\alpha, \beta, \lambda, \mu$ to represent indices with the range

$$\alpha, \beta, \lambda, \mu = 1, 2, \dots, h \quad (9)$$

corresponding to the alter-symmetric circuits on R , and

$$\alpha', \beta', \lambda', \mu' = h + 1, h + 2, \dots, 2h \quad (10)$$

to represent the *respective* symmetric indices belonging to circuits on R' .

The quantities

$$t_{\alpha\beta} = \frac{1}{2\pi i} (\tau_{\alpha\beta} - \tau_{\alpha'\beta} - \tau_{\alpha\beta'} + \tau_{\alpha'\beta'}) \quad (11)$$

play an important rôle. (Since $\tau_{\alpha\beta} = \tau_{\beta\alpha}$, we also have $t_{\alpha\beta} = t_{\beta\alpha}$.)

Using (8), we easily show that

$$v_{j'}(w) = -\overline{v_j(\bar{w})}, \quad (12)$$

$$\tau_{j'k'} = -\tau_{jk}, \quad (13)$$

where the bar denotes the conjugate complex quantity. It follows from (13) that $t_{\alpha\beta}$ is *real*, in fact

$$t_{\alpha\beta} = \Re \frac{1}{\pi i} (\tau_{\alpha\beta} - \tau_{\alpha'\beta}). \quad (14)$$

The determinant $|t_{\alpha\beta}|$ is readily seen to be non-vanishing, and hence the reciprocal matrix $T_{\alpha\beta}$ can be formed, where

$$T_{\alpha\lambda} t_{\lambda\mu} = \delta_{\alpha\mu} = \begin{cases} 1 & \text{if } \alpha = \mu, \\ 0 & \text{if } \alpha \neq \mu. \end{cases} \quad (15)$$

By the symmetry of $t_{\alpha\beta}$, we also have evidently $T_{\alpha\beta} = T_{\beta\alpha}$.

These are exactly the quantities $T_{\alpha\beta}$ which appear in the complementary terms of the general formula (2) for Green's function. Repetition of indices denotes summation over the range (9). If $h = 0$, *these complementary terms are simply not present*, and the expression for Green's function reverts to (1).

7. The formula (2) has the characteristic form for Green's function:

$$G(z, w) = -\Re \{ S(z, w) - S(z, \bar{w}) \}. \quad (16)$$

In the present instance, we have explicitly, by reference to (2) and (4),

$$S(z, w) = \log \theta(v(z, w); \tau) - T_{\alpha\beta} [v_\alpha(z) - v_{\alpha'}(z)] [v_\beta(w) - v_{\beta'}(w)]. \quad (17)$$

We have found it convenient to introduce also

$$Z(z, w) = \frac{\partial}{\partial \bar{z}} S(z, w), \quad P(z, w) = \frac{\partial^2}{\partial z \partial w} S(z, w). \quad (18)$$

The notation S, Z, P is intended to recall the elliptic functions $\log \sigma, \zeta, \wp$, to which the former reduce essentially when the Riemann surface \mathfrak{R} is of genus $p = 1$.

In the case of k arbitrary, but $h = 0$, the complementary terms disappear, as has been said, from the formula (17); we then have simply

$$S(z, w) = \log \theta(v(z, w); \tau). \quad (19)$$

By the second defining relation (18), with reference to the later explained notation (35), this gives

$$P(z, w) = \wp_{jk}(v(z, w))v'_j(z)v'_k(w), \quad (20)$$

where we leave implicit the dependence of our functions on the periods τ .

But if $h > 0$, we have, with complementary terms,

$$P(z, w) = \wp_{jk}(v(z, w))v'_j(z)v'_k(w) - T_{\alpha\beta}[v'_\alpha(z) - v'_{\alpha'}(z)][v'_\beta(w) - v'_{\beta'}(w)] \quad (21)$$

8. Let us denote by $\mathbf{H}(u, v)$, with the components $H_i(u, v)$ ($i = 1, 2, \dots, n$), the harmonic vector function on R with the boundary values $\mathbf{g}(z)$ on C .¹⁶ We can always express $\mathbf{H}(u, v)$ in the form

$$\mathbf{H}(u, v) = \Re \mathbf{F}(w), \quad w = u + iv. \quad (22)$$

Then the "vector Dirichlet integral"

$$D(\mathbf{H}) = \frac{1}{2} \int \int_R (\mathbf{H}_u^2 + \mathbf{H}_v^2) du dv = \frac{1}{2} \int \int_R \sum_{i=1}^n \left[\left(\frac{\partial H_i}{\partial u} \right)^2 + \left(\frac{\partial H_i}{\partial v} \right)^2 \right] du dv, \quad (22')$$

depends for its value only on \mathbf{g} and R : it is a *functional* of (\mathbf{g}, R) , which we denote by $A(\mathbf{g}, R)$. Upon this functional our entire method of solution is based; the required minimal surface M is determined by means of the principle

$$A(\mathbf{g}, R) = \min, \quad (22'')$$

where the range of the argument (\mathbf{g}, R) consists of all Riemann surfaces R having the topological form prescribed for M , together with all parametric representations \mathbf{g} of the given contours Γ on the boundaries C of R .

In "Two Contours" we established the following two fundamental formulas, governing our treatment of the Plateau problem for the *general topological case*:

$$A(\mathbf{g}, R) = D(\mathbf{H}) = \frac{1}{4\pi} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 P(z, \zeta) dz d\zeta, \quad (23)$$

$$\mathbf{F}'^2(w) = \sum_{i=1}^n F_i'^2(w) = \frac{1}{2\pi^2} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 P(z, w) P(\zeta, w) dz d\zeta. \quad (24)$$

In the case $h = 0$, these are explicitly, by reference to (20),

$$A(\mathbf{g}, R) = \frac{1}{4\pi} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 \wp_{jk}(v(z, \zeta)) v'_j(z) v'_k(\zeta) dz d\zeta, \quad (25)$$

$$\mathbf{F}'^2(w) = \frac{1}{2\pi^2} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 \wp_{jk}(v(z, w)) \wp_{km}(v(\zeta, w)) v'_j(z) v'_k(\zeta) v'_l(w) v'_m(w) dz d\zeta. \quad (26)$$

In the case $h > 0$, as is seen by reference to (21), these formulas acquire complementary terms \mathfrak{C} as follows:

$$\mathfrak{C}[A(\mathbf{g}, R)] = -\frac{1}{4\pi} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 T_{\alpha\beta} [v'_\alpha(z) - v'_{\alpha'}(z)] \times [v'_\beta(\zeta) - v'_{\beta'}(\zeta)] dz d\zeta, \quad (27)$$

$$\begin{aligned} \mathfrak{C}[\mathbf{F}'^2(w)] &= -\frac{1}{2\pi^2} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 T_{\alpha\beta} [v'_\alpha(\zeta) - v'_{\alpha'}(\zeta)] \times [v'_\beta(w) - v'_{\beta'}(w)] \wp_{jk}(v(z, w)) v'_j(z) v'_k(w) dz d\zeta \\ &\quad - \frac{1}{2\pi^2} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 T_{\alpha\beta} [v'_\alpha(z) - v'_{\alpha'}(z)] \times [v'_\beta(w) - v'_{\beta'}(w)] \wp_{jk}(v(\zeta, w)) v'_j(\zeta) v'_k(w) dz d\zeta \\ &\quad + \frac{1}{2\pi^2} \int_C \int_C [\mathbf{g}(z) - \mathbf{g}(\zeta)]^2 T_{\alpha\lambda} T_{\beta\mu} [v'_\alpha(z) - v'_{\alpha'}(z)] \times [v'_\beta(\zeta) - v'_{\beta'}(\zeta)] [v'_\lambda(w) - v'_{\lambda'}(w)] [v'_\mu(w) - v'_{\mu'}(w)] dz d\zeta. \end{aligned} \quad (28)$$

We proved in "*Two Contours*," for the case specified in its title, that the variational condition,

$$\delta A(\mathbf{g}, R) = 0, \quad (29)$$

in the problem $A(\mathbf{g}, R) = \min$ implied the condition

$$\mathbf{F}'^2(w) = 0, \quad (30)$$

characteristic of a minimal surface. The essential point in the proof was the use of an identity equivalent to the combined addition theorems of the elliptic functions¹⁷ ζ and \wp :

$$\wp(u + v) + \wp(u) + \wp(v) = [\zeta(u + v) - \zeta(u) - \zeta(v)]^2. \quad (31)$$

The identity actually used was the one resulting from this by the operator $\partial^2/\partial u \partial v$, being¹⁸

$$-\wp'(u+v)[\zeta(u) + \zeta(v)] + \wp(u+v)[\wp(u) + \wp(v)] + 2\pi i \frac{\partial \wp(u+v)}{\partial \tau} = \wp(u)\wp(v). \quad (32)$$

Here, however, the functions ζ , \wp were derived from the Jacobi function $\theta(u; \tau)$ with the characteristic $1/2, 1/2 (= -\theta_1(u; \tau))$ instead of from the Weierstrass function σ , as is understood in (31)—i.e., in (32),

$$\zeta(u) = \frac{\partial \log \theta(u; \tau)}{\partial u}, \quad \wp(u) = -\frac{\partial^2 \log \theta(u; \tau)}{\partial u^2}; \quad (33)$$

whereas in (31) ζ , \wp are defined by the same formulas with $\sigma(u)$ of periods $1, \tau$ in place of θ .

In the case of a general Riemann surface \mathfrak{R} of any finite genus p , we stated and proved for the first time, in "*Minimal Surfaces . . .*,"¹⁹ the following identity, generalizing (31):

$$\begin{aligned} & [\wp_{jk}(v(z, w)) + \wp_{jk}(v(z, \zeta)) + \wp_{jk}(v(\zeta, w))] v'_j(w) v'_k(w) \\ &= [\zeta_j(v(z, w)) - \zeta_j(v(z, \zeta)) - \zeta_j(v(\zeta, w))] [\zeta_k(v(z, w)) - \zeta_k(v(z, \zeta)) \\ &\quad - \zeta_k(v(\zeta, w))] v'_j(w) v'_k(w) + R(w), \end{aligned} \quad (34)$$

where $R(w)$ denotes some (undetermined) function of w alone, rational on \mathfrak{R} , i.e., uniform and with only polar singularities. Here, similar to (33), the standard notation is used:

$$\zeta_j(u; \tau) = \frac{\partial \log \theta(u; \tau)}{\partial u_j}, \quad \wp_{jk}(u; \tau) = -\frac{\partial^2 \log \theta(u; \tau)}{\partial u_j \partial u_k}; \quad (35)$$

in a moment, we shall employ also

$$\wp_{jkl}(u; \tau) = -\frac{\partial^3 \log \theta(u; \tau)}{\partial u_j \partial u_k \partial u_l}. \quad (36)$$

For the application to the Plateau problem, it was again necessary to apply to (34) the operator $\partial^2 / \partial z \partial \zeta$, which eliminated the unknown function $R(w)$, and gave²⁰

$$\begin{aligned} & \left\{ \wp_{jk}(v(z, \zeta)) [-\zeta_m(v(z, w)) + \zeta_m(v(\zeta, w))] \right. \\ & \quad + \wp_{kl}(v(z, \zeta)) \wp_{jm}(v(z, w)) + \wp_{jm}(v(z, \zeta)) \wp_{kl}(v(\zeta, w)) \\ & \quad \left. + 2\pi i \frac{\partial \wp_{jk}(v(z, \zeta))}{\partial \tau_{lm}} \right\} v'_i(z) v'_k(\zeta) v'_l(w) v'_m(w) \\ &= \wp_{ji}(v(z, w)) \wp_{km}(v(\zeta, w)) v'_j(z) v'_k(\zeta) v'_l(w) v'_m(w). \end{aligned} \quad (37)$$

The summation convention as to repeated indices applies to $j, k, l, m = 1, 2, \dots, p$.

10. With the use of this identity, we proved in "*Minimal Surfaces . . .*" that also for the case of any number k of boundaries of R or of M , provided that the genus $h = 0$, the variational equation (29) of $A(g, R)$ implies the condition (30) for a minimal surface.

One of the main features of the forthcoming Annals paper is the easy extension of this proof to the case $h > 0$.²¹ This requires only a discussion of the complementary terms, as expressed by the formulas (27), (28), and is a perfectly routine matter, not involving any such rather deep-lying identity as (37). Indeed, the full details are presented in the next section, no. 11, of this note.

It thus becomes apparent that *all the essential difficulties of the more general case are already disposed of with the case $h = 0$* , provided that the number k of given contours is arbitrary. That this is so depends essentially on the fact that the genus p of the complete Riemann surface \mathfrak{R} , as expressed by the formula (3) $p = 2h + k - 1$, takes an arbitrary value as soon as k is arbitrary, even if h be restricted to the value zero. The new feature associated with $h > 0$ is that alter-symmetric circuits appear on \mathfrak{R} , which give rise to the complementary terms in Green's function, but, as we shall see in the next section, the effect of these is very easy to trace. The principal term of Green's function is the one involving Ω , and thereby the θ -function on \mathfrak{R} . It was the treatment of this term in application to the Plateau problem which required the identity (37).

11. We now give in detail the calculations involving the complementary terms of Green's function.

These produce the complementary terms of $A(g, R)$, as given in (27). Putting aside the factor $1/4\pi$, we write for the kernel of the integral which expresses $\mathfrak{C}[A(g, R)]$:

$$K = -T_{\alpha\beta}[v'_\alpha(z) - v'_{\alpha'}(z)][v'_\beta(\zeta) - v'_{\beta'}(\zeta)]. \quad (38)$$

As remarked in "*Minimal Surfaces . . .*" (p. 117), the Riemann surface \mathfrak{R} can always be given a conformally equivalent form on which any pre-assigned point w of \mathfrak{R} becomes a branch-point of first order (like that of \sqrt{z}). If this branch-point moves, then the normal integrals v of the first kind undergo the variation (loc. cit., formula 15)

$$\delta v_j(z) = -v'_j(w)v'_k(w)\zeta_k(v(z, w)). \quad (39)$$

This induces on the periods τ_{jk} of v the variation (loc. cit., formula 16)

$$\delta\tau_{jk} = 2\pi i v'_j(w)v'_k(w), \quad (40)$$

since the period of $\zeta_k(v(z, w))$ when z traverses a circuit B_m is known to be $-2\pi i \delta_{km}$.

We have then, by applying δ to (38),

$$\begin{aligned}\delta K = & -T_{\alpha\beta}[\delta v'_\alpha(z) - \delta v'_{\alpha'}(z)][v'_\beta(\zeta) - v'_{\beta'}(\zeta)] \\ & - T_{\alpha\beta}[v'_\alpha(z) - v'_{\alpha'}(z)][\delta v'_\beta(\zeta) - \delta v'_{\beta'}(\zeta)] \\ & - \delta T_{\alpha\beta}[v'_\alpha(z) - v'_{\alpha'}(z)][v'_\beta(\zeta) - v'_{\beta'}(\zeta)].\end{aligned}\quad (41)$$

Differentiating the variational formula (39) as to z , we get

$$\delta v'_\alpha(z) - \delta v'_{\alpha'}(z) = [v'_\alpha(w) - v'_{\alpha'}(w)]\wp_{jk}(v(z, w))v'_j(z)v'_k(w). \quad (42)$$

Similarly,

$$\delta v'_\beta(\zeta) - \delta v'_{\beta'}(\zeta) = [v'_\beta(w) - v'_{\beta'}(w)]\wp_{jk}(v(\zeta, w))v'_j(\zeta)v'_k(w). \quad (43)$$

We recall the commutativity of differentiation and variation, a standard fact of the calculus of variations.

Apply to (15) the operator δ ; then multiply by $T_{\beta\mu}$ and sum as to μ ; this gives

$$\delta T_{\alpha\beta} = -T_{\alpha\lambda}T_{\beta\mu}\delta t_{\lambda\mu}. \quad (44)$$

By (11) and (39), we easily find

$$\delta t_{\lambda\mu} = [v'_\lambda(w) - v'_{\lambda'}(w)][v'_\mu(w) - v'_{\mu'}(w)]; \quad (45)$$

hence

$$\delta T_{\alpha\beta} = -T_{\alpha\lambda}T_{\beta\mu}[v'_\lambda(w) - v'_{\lambda'}(w)][v'_\mu(w) - v'_{\mu'}(w)]. \quad (46)$$

Combining (41), (42), (43) and (46), we obtain, with change of indices,

$$\begin{aligned}\delta K = & -T_{\alpha\beta}[v'_\alpha(\zeta) - v'_{\alpha'}(\zeta)][v'_\beta(w) - v'_{\beta'}(w)]\wp_{jk}(v(z, w))v'_j(z)v'_k(w) \\ & - T_{\alpha\beta}[v'_\alpha(z) - v'_{\alpha'}(z)][v'_\beta(w) - v'_{\beta'}(w)]\wp_{jk}(v(\zeta, w))v'_j(\zeta)v'_k(w) \\ & + T_{\alpha\lambda}T_{\beta\mu}[v'_\alpha(z) - v'_{\alpha'}(z)][v'_\beta(\zeta) - v'_{\beta'}(\zeta)][v'_\lambda(w) - v'_{\lambda'}(w)] \\ & \quad [v'_\mu(w) - v'_{\mu'}(w)].\end{aligned}\quad (47)$$

By comparison with (28), this is seen to be *proportional exactly to the kernel of the integral which expresses* $\mathfrak{C}[\mathbf{F}'^2(w)]$.

Thus it results that

$$\delta \mathfrak{C}[A(\mathbf{g}, R)] = \frac{\pi}{2} \mathfrak{C}[\mathbf{F}'^2(w)]. \quad (48)$$

But in "*Minimal Surfaces . . .*" we proved a precisely similar relation for the *principal terms* of $A(\mathbf{g}, R)$ and $\mathbf{F}'^2(w)$, as they appear in (25), (26), i.e.,

$$\delta A(\mathbf{g}, R) = \frac{\pi}{2} \mathbf{F}'^2(w), \quad \text{for } h = 0. \quad (49)$$

By adding this to the preceding formula, it follows that also in the *general case* $h > 0$, we have

$$\delta A(\mathbf{g}, R) = \frac{\pi}{2} \mathbf{F}'^2(w). \quad (50)$$

Consequently, in the general case likewise, the variational equation of $A(g, R)$ implies the condition $F'^2(w) = 0$, which characterizes as a minimal surface the one defined by the harmonic vector $H(u, v)$ on R with the boundary values g .

12. With this, the Plateau problem is solved in the generality stated at the beginning of this note. For the attainment of the minimum of $A(g, R)$ has been established many times in the author's previous papers on the basis of the compactness of the argument range $[(g, R)]$ and the lower semi-continuity of the functional A . We have only to prevent degeneration of the required minimal surface. This is done by means of certain simple sufficient conditions first given in the author's paper "*New Results*,"²² which express that the lower bound of the areas of all *proper* surfaces of topological type r bounded by the given contours Γ is strictly less than the similar lower bound for *improper* surfaces of that type.²³

In the following note an alternative method is presented of treating the general topological form of the Plateau problem, which uses Green's function in an intrinsic way, without employing for it any explicit formula—a circumstance which enables us to avoid many of the preceding calculations. This method proceeds in the same way in all cases, entirely regardless of the particular topological structure of the required minimal surface.

¹ The definition of r is the maximum number of circuits, no linear combination of which separates the surface. For a two-sided surface, $r = 2h$. For a one-sided surface, r may be odd or even; examples: Möbius surface with h handles, $r = 2h + 1$; Klein surface with h handles, $r = 2h + 2$. See Hilbert and Cohn-Vossen, *Anschauliche Geometrie*, 1932.

² J. Douglas, *Bull. Amer. Math. Soc.*, **36**, 50 (1930).

³ In the following publications, respectively: *Trans. Amer. Math. Soc.*, **33**, 263–321 (1931); *Journ. Math. Phys.*, **10**, 310–359 (1931); *Trans. Amer. Math. Soc.*, **34**, 731–756 (1932). The second of these will be referred to as "*Two Contours*."

⁴ Loc. cit., p. 324. See Formulas (23), (24) of the present note.

⁵ J. Douglas, "*Some New Results in the Problem of Plateau*," *Journ. Math. Phys.*, **15**, 55–64 (Feb., 1936); "*Minimal Surfaces of General Topological Structure with any Finite Number of Assigned Boundaries*," *Ibid.*, **15**, 105–123 (June, 1936). These papers will be referred to respectively as "*New Results*" and "*Minimal Surfaces . . .*"

⁶ These PROCEEDINGS, **22**, 367–372 (June, 1936).

⁷ R. Courant, *Ann. Math.*, **38**, 679–724 (1937).

⁸ *Rendiconti Lincei*, **24**, 333–339, 393–398 (Nov., Dec., 1936).

⁹ Specifically, the new items included in the present note are formulas (2), (27), (28) and §11.

¹⁰ As far as I know, an explicit formula such as (2) has not hitherto been given for the Green's function of a general Riemann surface. (1) was given in "*Minimal Surfaces . . .*" as formula (2) (equivalent to formulas (1) and (4) of the present note).

¹¹ For abelian integrals and algebraic geometry, see, throughout this note, E. Picard, *Traité d'Analyse*, 2, chaps. 14–16 (1925), and Severi-Löffler, *Vorlesungen über Algebraische Geometrie*, 1921.

¹² See the definition, immediately after formula (8).

¹³ See the author's previously cited paper in *Trans. Amer. Math. Soc.*, **34**, 731–756 (1932). By means of the relation just described between one- and two-sided surfaces,

the case $r = 1$ of a Möbius surface with any finite number k of boundaries can be referred to the case $k = 0$, where there are $2k$ boundaries, symmetric in pairs. Accordingly, the case $r = 1$ can likewise be treated with the simpler formula (1) for Green's function.

¹⁴ See H. F. Baker, "Abel's Theorem and . . . Theta Functions," 1897, chapter X.

¹⁵ In the notation of symmetric indices j, j' which we introduce immediately hereafter, we suppose also $\alpha_j = \alpha_{j'}$, $\beta_j = \beta_{j'}$.

The α, β which denote the characteristic of the θ -series have, of course, nothing to do with the summation indices of formula (2). For the rest of this note, α, β will be used exclusively in the latter meaning.

¹⁶ Systematically, the letters $\mathbf{H}, \mathbf{g}, \mathbf{F}$ in bold-faced type will denote *vectors* with n components.

¹⁷ As notation, the function ζ is easily distinguished from the argument ζ which appears in (23) et seq.

¹⁸ Equivalent to (7.1) of "Two Contours."

¹⁹ Loc. cit., formula (41).

²⁰ "Minimal Surfaces . . .," formula (33).

²¹ Loc. cit., §12.

²² Theorems I, II, and footnote 9; see as well the similarly numbered theorems of the forthcoming *Annals* paper.

²³ That is, surfaces S bounded by Γ which are expressible in the form $S = S_1 + S_2 + \dots + S_m$ where the respective characteristics obey the condition $r_1 + r_2 + \dots + r_m \leq r$ and either $m > 1$ or the relation $<$ holds.

GREEN'S FUNCTION AND THE PROBLEM OF PLATEAU

BY JESSE DOUGLAS

BROOKLYN, N. Y.

Communicated July 1, 1938

1. The general topological form of the problem of Plateau requires the determination of a minimal surface M of any given finite genus h or characteristic r , either character of orientability (one- or two-sided), and with any finite number k of boundaries Γ , assigned in form and position in n -dimensional euclidean space.¹

The author has based his solution of this problem² on the functional

$$A(\mathbf{g}, R) = D(\mathbf{H}) = \int \int_R \frac{1}{2} (E + G) du dv = \frac{1}{2} \int \int_R (\mathbf{H}_u^2 + \mathbf{H}_v^2) du dv. \quad (1)$$

Here R denotes any Riemann surface having the topological form prescribed for the minimal surface M ; \mathbf{g} denotes any parametric representation of the contours Γ on the boundaries C of R . $\mathbf{H}(u, v)$ is the harmonic vector function on R with the values \mathbf{g} on C ; E, F, G denote the first fundamental quantities of the surface $\mathbf{x} = \mathbf{H}(u, v)$; $\mathbf{g}, \mathbf{H}, \mathbf{x}$ are, throughout, *vectors*

of n components; D , applied to a vector, denotes one-half the sum of the Dirichlet integrals of its components.

A minimal surface M is one definable as a *harmonic* and *conformal* image of a Riemann surface R , i.e., in the form

$$\mathbf{x} = \mathbf{H}(u, v), \quad (2)$$

with

$$E = G, \quad F = 0. \quad (3)$$

These express the variational condition in the problem of the surface of least area with given boundaries.

To include one-sided minimal surfaces \bar{M} in our theory, we employ a two-sided covering surface in two-one point correspondence with \bar{M} .³

2. *Riemann Surfaces*.—The Riemann surface R may always be considered as one of the conjugate halves (semi Riemann surface) of a *symmetric* Riemann surface \mathfrak{R} . The symmetric property of \mathfrak{R} consists in the existence of an inversely conformal transformation T which associates the points of \mathfrak{R} in pairs P, \bar{P} , called conjugate or symmetric. \mathfrak{R} may always be represented as the Riemann surface of a real algebraic curve $\mathfrak{A}: P(x, y) = 0$ (real coefficients), and T may then be interpreted as the interchange of conjugate complex points $(x, y), (\bar{x}, \bar{y})$. The points fixed under T constitute the real branches C of \mathfrak{A} . \mathfrak{A} is subject to any real birational transformation without essential change in \mathfrak{R} ; i.e., \mathfrak{R} remains conformal to itself.

The complete complex manifold \mathfrak{A} may be represented by writing in $P(x, y) = 0$,

$$x = x_1 + ix_2, \quad y = y_1 + iy_2, \quad (4)$$

giving

$$P(x_1 + ix_2, y_1 + iy_2) = 0, \quad (5)$$

which, by separation of real and imaginary parts, gives two equations

$$P_1(x_1, x_2, y_1, y_2) = 0, \quad P_2(x_1, x_2, y_1, y_2) = 0. \quad (6)$$

These represent in the ordinary four-dimensional space (x_1, x_2, y_1, y_2) a two-dimensional manifold \mathfrak{S} , which may be taken as one of the forms of the Riemann surface of \mathfrak{A} . The *section* of \mathfrak{S} by the real (x_1, y_1) -plane gives the real branches of \mathfrak{A} . The *orthogonal projection* of \mathfrak{S} on the complex x - and y -planes gives the Riemann surfaces $\mathfrak{R}_x, \mathfrak{R}_y$ in the standard form of many-sheeted surfaces over these planes. $\mathfrak{S}, \mathfrak{R}_x, \mathfrak{R}_y$ are all in conformal correspondence by this projection, and are therefore equivalent for all our purposes.

If we suppose that the real branches of \mathfrak{A} separate the Riemann surface \mathfrak{S} , or $\mathfrak{R} = \mathfrak{R}_x$ or \mathfrak{R}_y ,⁴ then either of the conjugate halves may be taken as R . The boundaries of R are then precisely these real branches C ; and R ,

previously called a semi Riemann surface, may be referred to simply as a Riemann surface, except that it has boundaries while the complete Riemann surface \mathfrak{R} is closed.

3. In the solutions previously given by the author for the general topological form of the Plateau problem, the analytic procedure was based on an explicit formula for the Green's function of R in terms of θ -functions and abelian integrals on the complete Riemann surface \mathfrak{R} .⁵

The Green's function $G(P_1, P_2)$ of R is uniquely defined by the following properties. As a function of the point P_1 , $G(P_1, P_2)$ is: (i) uniform on R , (ii) harmonic on R , (iii) logarithmically singular for $P_1 = P_2$, (iv) equal to zero when P_1 is on the boundary C of R . Property (iii) means that for P_1 in the vicinity of P_2 , we have

$$G(P_1, P_2) = -\log P_1 P_2 + G_1(P_1, P_2) \quad (7)$$

where $G_1(P_1, P_2)$ is regular for $P_1 = P_2$. We may recall also the well-known symmetry property

$$G(P_1, P_2) = G(P_2, P_1). \quad (8)$$

The purpose of this note is to present the essential features of a simpler method of treatment, which uses Green's function in an intrinsic way, without employing for it any explicit expression. This method proceeds in the same way in all cases, regardless of the particular topological form of R or M .

A detailed presentation will appear elsewhere under the same title.

4. Equivalent to the defining formulas (2), (3) of a minimal surface M are the following:

$$\mathbf{x} = \mathbf{H}(Q), \quad (9)$$

$$\frac{\partial \mathbf{H}(Q)}{\partial \xi} \cdot \frac{\partial \mathbf{H}(Q)}{\partial \eta} = 0. \quad (10)$$

Here Q denotes an arbitrary interior point of R , and ξ, η are any two perpendicular directions on R at Q .

For (10) expresses that any two perpendicular directions on R are converted into perpendicular directions on M , which is sufficient to secure the *conformal* nature of the correspondence between the two surfaces. Indeed, the conditions (3) of conformality are merely two particular cases of (10), namely, for ξ, η coincident with the u, v directions, and with their angle-bisectors.

M will be bounded by given contours Γ if the values of $\mathbf{H}(Q)$ on the boundaries C of R , i.e.,

$$\mathbf{x} = \mathbf{g}(P), \quad (11)$$

form a parametric representation of Γ .

5. It will convey a sufficiently typical impression of the contents of our detailed paper if we simply reproduce its three fundamental formulas, and quote its main theorem. The latter provides an explicit construction, in both geometric and analytic form, of a special variation of the Riemann surface R , which realizes the variational formula of Green's function given hereafter as (16).

We imagine that the variation of R to R_ϵ takes place by simultaneous variation of the individual points: P_1 to $P_1(\epsilon)$, P_2 to $P_2(\epsilon)$, etc. Then Green's function $G(P_1, P_2)$, which depends on the form of the Riemann surface R_ϵ and the position of the points $P_1(\epsilon)$, $P_2(\epsilon)$, becomes, for given points P_1, P_2 on R , a function of ϵ :

$$G_\epsilon(P_1(\epsilon), P_2(\epsilon)) = G(\epsilon; P_1, P_2). \quad (12)$$

We define the variation of $G(P_1, P_2)$ by the usual formula⁷

$$\delta G(P_1, P_2) = \frac{\partial}{\partial \epsilon} G(\epsilon; P_1, P_2) \Big|_{\epsilon=0}. \quad (13)$$

6. *Formulas.*—The three basic formulas referred to are the following:

$$A(\mathfrak{g}, R) = \frac{1}{8\pi} \int_C \int_C [\mathfrak{g}(P_1) - \mathfrak{g}(P_2)]^2 \frac{\partial^2 G(P_1, P_2)}{\partial n_1 \partial n_2} ds_1 ds_2. \quad (14)$$

$$\frac{\partial H(Q)}{\partial \xi} \cdot \frac{\partial H(Q)}{\partial \eta} = - \frac{1}{16\pi^2} \int_C \int_C [\mathfrak{g}(P_1) - \mathfrak{g}(P_2)]^2 \frac{\partial^2}{\partial n_1 \partial n_2} \left\{ \frac{\partial G(P_1, Q)}{\partial \xi} \frac{\partial G(P_2, Q)}{\partial \eta} + \frac{\partial G(P_1, Q)}{\partial \eta} \frac{\partial G(P_2, Q)}{\partial \xi} \right\} ds_1 ds_2. \quad (15)$$

$$\delta G(P_1, P_2) = \frac{\partial G(P_1, Q)}{\partial \xi} \frac{\partial G(P_2, Q)}{\partial \eta} + \frac{\partial G(P_1, Q)}{\partial \eta} \frac{\partial G(P_2, Q)}{\partial \xi}. \quad (16)$$

Here $\partial/\partial n_1$, $\partial/\partial n_2$ denote differentiation in the direction of the interior normal to C at P_1 , P_2 , respectively.

The bearing of these formulas on the Plateau problem is apparent. For by combining them, we evidently get

$$\delta A(\mathfrak{g}, R) = - 2\pi \frac{\partial H(Q)}{\partial \xi} \cdot \frac{\partial H(Q)}{\partial \eta}. \quad (17)$$

In this, we use the readily established fact of the commutativity of the operators δ and $ds_1 ds_2 \partial^2/\partial n_1 \partial n_2$.

It follows that the variational condition

$$\delta A(\mathfrak{g}, R) = 0, \quad (18)$$

associated with our fundamental minimum principle

$$A(\mathfrak{g}, R) = \min, \quad (19)$$

is equivalent exactly to the condition (10) for a minimal surface.

The variational formula (16) is thus seen to serve the same purpose, in the present method of solution, as was served in our previous method by the basic identity in θ -functions.* For the purpose of that identity was precisely to bring about the equivalence of the variational condition on the functional $A(\mathfrak{g}, R)$ to the condition for a minimal surface.

In connection with the formula (14), we may remark that in the simplest case, where R is the unit circle and C its circumference, on which P_1, P_2 with polar angles θ, φ are any two points, we have by standard formulas,

$$\frac{\partial^2 G(P_1, P_2)}{\partial n_1 \partial n_2} = \frac{1}{2 \sin^2 \frac{\theta - \varphi}{2}}. \quad (20)$$

The general formula (14) then reverts to the particular form⁹

$$A(\mathfrak{g}) = \frac{1}{4\pi} \int_C \int_C \frac{[\mathfrak{g}(\theta) - \mathfrak{g}(\varphi)]^2}{4 \sin^2 \frac{\theta - \varphi}{2}} d\theta d\varphi, \quad (21)$$

on which we based our original solution for a single given contour and a simply-connected minimal surface.

7. We may now quote our main theorem, and then conclude with some explanatory figures and remarks.

VARIATIONAL THEOREM CONCERNING GREEN'S FUNCTION

THEOREM. Let \mathfrak{A} denote any real algebraic curve, on whose Riemann surface, \mathfrak{B} or \mathfrak{B} , the points Q, \bar{Q} are any two conjugate imaginary points.

Let the tangent lines t, \bar{t} to \mathfrak{A} at Q, \bar{Q} intersect in the real point O . Choose a reference triangle with one vertex at O ; then in homogeneous line coördinates u, v, w , the equation of \mathfrak{A} will evidently have the form

$$\mathfrak{A}: (au^2 + buv + cv^2) K(u, v) + wL(u, v, w) = 0,$$

where

$$t, \bar{t}: au^2 + buv + cv^2 = 0$$

represents the conjugate imaginary tangents t, \bar{t} . K and L are homogeneous polynomials.

Construct now the family of curves with parameter ϵ ,

$$\mathfrak{A}_\epsilon: [(a + a'\epsilon)u^2 + (b + b'\epsilon)uv + (c + c'\epsilon)v^2] K(u, v) + wL(u, v, w) = 0,$$

where a', b', c' are fixed but arbitrary real coefficients.

Then by rectilinear projection \mathbb{P} from O , the Riemann surfaces \mathfrak{B} , \mathfrak{B}_ϵ of \mathfrak{A} and \mathfrak{A}_ϵ are set into one-one continuous and conformal correspondence: P to $P(\epsilon)$, except in the immediate vicinity of Q , \bar{Q} . In particular, the real branches C , C_ϵ of \mathfrak{A} and \mathfrak{A}_ϵ are thereby set into one-one continuous correspondence. This depends on the circumstance that all the real tangents from O to \mathfrak{A}_ϵ —which are defined by the real factors of the equation

$$K(u, v) = 0$$

—remain invariant, since this equation is independent of ϵ .

In fact, for the same reason, all the tangents, real and imaginary, from O to \mathfrak{A}_ϵ , except t_ϵ , \bar{t}_ϵ , remain fixed. These, however, vary with ϵ ; and t_ϵ , for instance, intersects the Riemann surface \mathfrak{B} of \mathfrak{A} in two points near to Q ,¹⁰ which, as ϵ passes through the value zero from positive to negative, always enter Q from two opposite directions α , α' and leave in the perpendicular opposite directions β , β' . Let the angle-bisectors of $\alpha\beta$, $\alpha'\beta'$ be the perpendicular directions ξ , η .

Denote by $G(P_1, P_2)$ the Green's function of either conjugate semi-surface R of the symmetric Riemann surface \mathfrak{B} .¹¹ Then, under the precedingly described variation of the form of \mathfrak{B} and the position of the points P_1 , P_2 , we have for Green's function the variational formula

$$(V): \quad \delta G(P_1, P_2) = \frac{\partial G(P_1, Q)}{\partial \xi} \frac{\partial G(P_2, Q)}{\partial \eta} + \frac{\partial G(P_1, Q)}{\partial \eta} \frac{\partial G(P_2, Q)}{\partial \xi}$$

(apart from an inessential numerical factor).

Finally, the directions ξ , η can be made to coincide with any preassigned perpendicular directions at Q , by proper choice of a' , b' , c' .

This theorem seems of particular interest for its interplay of fundamental analytic and geometric entities, as well as for its direct application to the Plateau problem.

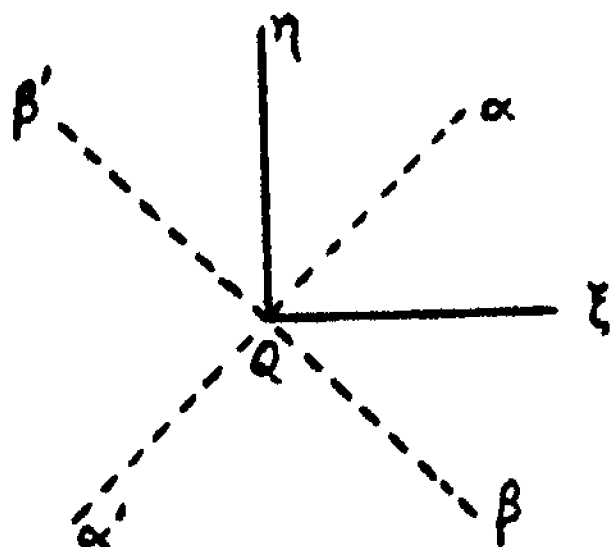


FIGURE 1

8. Figure 1 shows the directions α , α' ; β , β' ; ξ , η ; referred to in the preceding statement.

Figure 2 illustrates the real branches of the curve \mathfrak{A} drawn full, and of the varied curve \mathfrak{A}_ϵ drawn dotted. The real tangents from O are indicated by full drawn lines. One other projecting line from O is drawn dotted, and upon it corresponding points P , P_ϵ are indicated.

It is evident how the fact that \mathfrak{A}_ϵ remains always tangent to the same real lines through O conditions the one-to-one nature of the correspondence between the real branches of \mathfrak{A} and \mathfrak{A}_ϵ established by the projection from O .¹² For, otherwise, either \mathfrak{A} or \mathfrak{A}_ϵ would protrude outside one of the real

tangents to the other from O , and then the protruding arc, say of \mathbf{A}_1 , could have no corresponding real arc on \mathbf{A} .

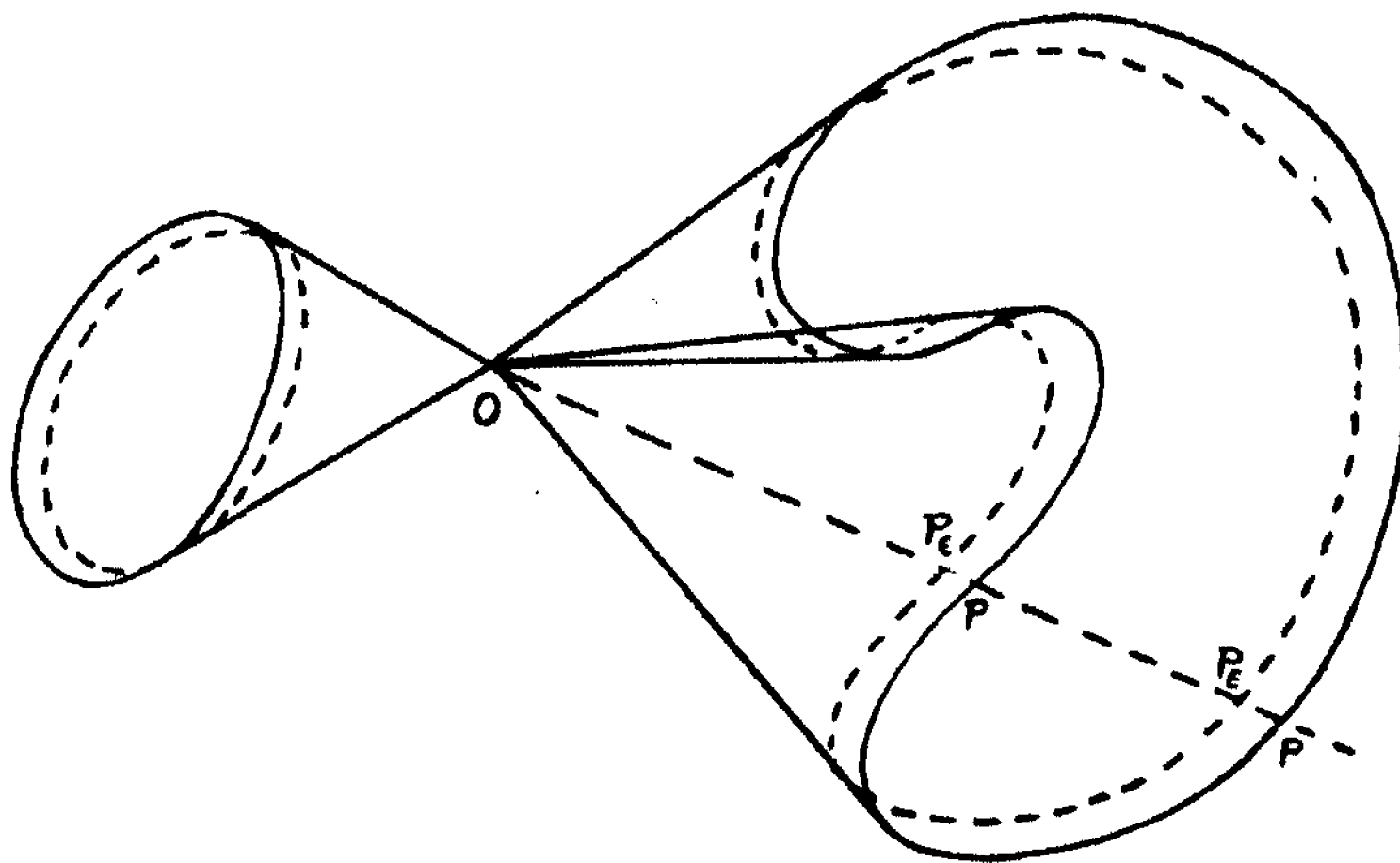


FIGURE 2

REFERENCES

J. Douglas:

1. "Some New Results in the Problem of Plateau," *Jour. Math. Phys.*, **15**, 55-64 (1936).
2. "Minimal Surfaces of General Topological Structure with any Finite Number of Assigned Boundaries," *Ibid.*, **15**, 105-123 (1936).
3. "Solution of the Problem of Plateau," *Trans. Amer. Math. Soc.*, **33**, 263-321 (1931).
4. "The Problem of Plateau," *Bull. Amer. Math. Soc.*, **39**, 227-251 (1933).
5. "The Problem of Plateau for Two Contours," *Jour. Math. Phys.*, **10**, 310-359 (1931).
6. "One-Sided Minimal Surfaces with a Given Boundary," *Trans. Amer. Math. Soc.*, **34**, 731-756 (1932).
7. "Crescent-Shaped Minimal Surfaces," these PROCEEDINGS, **19**, 192-199 (1933).
8. "Minimal Surfaces of Higher Topological Structure," *Ann. Math.* (in press).
9. The foregoing note in these PROCEEDINGS, with the same title as [8].

¹ This general form of the Plateau problem was first formulated by the author in *Bull. Amer. Math. Soc.*, **36**, 50 (1930).

² In [1], [2], [8], [9] of the list of references at the end of this paper, citations from which will be made by numbers in square brackets. The other papers in the list deal with important particular cases leading up to the general one: one contour [3], [4]; two non-intersecting contours [5]; Möbius strip [6]; two contours intersecting in a single point [7]. In these particular cases, except for [6], where the characteristic r is unity, the required minimal surfaces were required to be of genus $h = 0$.

³ Cf. [6], p. 734; [1], p. 61.

⁴ Symmetric Riemann surface of the first kind; see F. Klein, *Über Riemanns Theorie der Algebraischen Funktionen und ihrer Integrale*, 1882.

⁵ [8], formula (7.17); [9], formula (2); for the case $h = 0$, [2], formula (2).

⁶ The dot denotes the scalar product of vectors. The exponent two (formulas 14, 15) will denote the scalar product of a vector by itself, i.e., the sum of the squares of its components.

⁷ Except that we interpret δ as a *derivative*, rather than a differential, as is more customary.

⁸ Formulas (41) and (33) of [2], reproduced as (34) and (37) of the foregoing note in the present issue of these PROCEEDINGS. This is for a general topological form of the Riemann surface \mathfrak{R} . With increasing complexity of \mathfrak{R} , the appropriate identity involved successively: algebraic functions, trigonometric functions, elliptic functions, θ -functions. See, respectively [4], p. 243; [7], formula (5.4); [5], formula (7.1); [2], formulas (41), (33). In [7], the functions actually appearing are hyperbolic, due to the rotation through a right angle of the parallel strip representing the Riemann surface R .

⁹ Given in [3], and in preceding abstracts in *Bull. Amer. Math. Soc.*, 36, 50 (1930).

¹⁰ We suppose that the contact of the tangents l, \bar{l} to \mathfrak{A} at Q, \bar{Q} , respectively, is ordinary two-point contact. Higher contact can always be avoided by a preliminary birational transformation.

¹¹ Or its conformally equivalent orthogonal projections $\mathfrak{R} = \mathfrak{R}_x$ or \mathfrak{R}_y (see art. 2). The notation is supposed arranged so that the semi-surface R contains the point Q .

¹² We may again remark that this one-to-one character breaks down in the vicinity of the points of contact Q, \bar{Q} of the tangents l, \bar{l} . Otherwise, it extends beyond the real branches to the rest of the Riemann surfaces involved, with deletion of the stated neighborhoods.

THE MOST GENERAL FORM OF THE PROBLEM OF PLATEAU

BY JESSE DOUGLAS

BROOKLYN, New York

Communicated June 28, 1938

1. The method of the preceding note—cited hereafter as Note II—applies with practically no modification to the following “most general formulation” of the problem of Plateau, which, I believe, is given here explicitly for the first time.

PROBLEM P. *Given any riemannian manifold R in the most general sense of the term, i.e., any two-dimensional connected topological variety for which there is defined in the neighborhood of each point a local conformal representation on a circle.¹ R may then have any finite or infinite number of boundaries, and any topological structure whatever, i.e., any finite or infinite type of connectivity. It may also have either character of orientability, i.e., one- or two-sidedness.²*

Given also any point-set Γ in n -dimensional euclidean space which is a topological image of the total boundary C of R . Γ may consist of any finite or infinite number of Jordan curves, together with their limit points; or it may be

some more general type of point-set. A definite sense of description is associated with each component of Γ , carried over from C , which may be supposed to be oriented so that R is on the left.

To determine the existence of a minimal surface M topologically equivalent to R and bounded by Γ .

2. The exact analytic meaning of the requirement expressed in the last sentence is the same as in art. 4 of Note II.

If R has only a finite number k of boundaries and finite topological characteristic r , then we have the problem dealt with in Note II. This most important case of Problem P we shall call Problem P_0 .

In Problem P_0 , R is the Riemann semi-surface of a real *algebraic* curve; in Problem P, R is the Riemann semi-surface of a real *analytic* curve \mathbf{A} . This means the manifold that is obtained from the Riemann surface \mathbf{R} associated with the equation $y = f(x)$ of the analytic curve \mathbf{A} by combining into a single geometric element each pair of conjugate complex points $(x, y), (\bar{x}, \bar{y})$ of \mathbf{A} . That \mathbf{A} is *real* means that the power-series elements of the analytic function $y = f(x)$ occur in conjugate complex pairs, i.e., with conjugate complex centers x_0, \bar{x}_0 and coefficients a_m, \bar{a}_m .

The *boundary* C of R consists of the *real branches* of \mathbf{A} , and R is two- or one-sided according as C does or does not separate \mathbf{R} .

Thus, if \mathbf{A} is the curve

$$y^2 = \sin x \quad (1)$$

we have the problem of a minimal surface M topologically equivalent to a sphere with an infinite number of perforations that converge to a single point p , and where the prescribed boundary Γ of M consists of an infinite number of Jordan curves in space which converge to a single point P .

If, as another example, \mathbf{A} is

$$y^2 = (x^2 + 1) \sin x, \quad (2)$$

then the form required for M is that of a Möbius surface with an infinite number of boundaries, which have assigned positions Γ in space, of the same type of distribution as before.

If \mathbf{A} is

$$y^2 = \sin x \cosh x, \quad (3)$$

then the form of M is that of a one-sided surface with an infinite number of handles or branch-cuts converging to a point and an infinite number of given boundaries Γ converging to a point.

3. We introduce the following notation and definitions, and quote also the simple preliminary theorems (7°), (12°).

(1°) \mathfrak{g} shall denote any representation of the given complete boundary Γ as topological image of the total boundary C of R .

(2°) *Definition.*

$$A(\mathfrak{g}, R) = \frac{1}{2} \int \int_R (\mathbf{H}_u^2 + \mathbf{H}_v^2) du dv, \quad (4)$$

where $\mathbf{H}(u, v)$ is the harmonic vector function on R whose boundary values are \mathfrak{g} .

(3°) \mathfrak{U} shall denote the class of all riemannian manifolds topologically equivalent to R .

(4°) \mathfrak{U}' shall denote all riemannian manifolds R' which are the limit of a sequence of manifolds R_m of \mathfrak{U} , without belonging to \mathfrak{U} themselves.

Any such manifold R' either consists of a finite or infinite number of separate parts, or, if consisting of a single component, is of lower topological type than R . If the equation of the analytic curve \mathbf{A}' corresponding to R' is $F(x, y) = 0$, then, accordingly, either $F(x, y)$ is reducible, separating into a finite or infinite number of factors, or else it acquires new conjugate complex multiple points by coalescence of branch-points of R .

\mathfrak{U}' may be regarded as the "frontier" of \mathfrak{U} in the "space" of all riemannian manifolds.

(5°) *Definition.*

$$d(\Gamma, \mathfrak{U}) = \min A(\mathfrak{g}, R), \quad (5)$$

for all riemannian manifolds R belonging to \mathfrak{U} , and all topological representations \mathfrak{g} of Γ as image of the boundary C of R . Throughout, we understand "min" in the sense of *lower bound*, without prejudice of the question as to whether the minimum is attained or not.

The value of $d(\Gamma, \mathfrak{U})$ may be either a finite positive number, or $+\infty$, depending on the case.

(6°) *Definition.*

$$d(\Gamma, \mathfrak{U}') = \min A(\mathfrak{g}, R'), \quad (6)$$

for all manifolds R' of \mathfrak{U}' , and all topological representations \mathfrak{g} of Γ as image of the total boundary C of all components of R' .

(7°) **THEOREM.** *In every case,*

$$d(\Gamma, \mathfrak{U}) \leq d(\Gamma, \mathfrak{U}'). \quad (7)$$

(8°) *Definition.* For $d(\Gamma, \mathfrak{U})$ finite,

$$e(\Gamma, \mathfrak{U}) = d(\Gamma, \mathfrak{U}') - d(\Gamma, \mathfrak{U}). \quad (8)$$

By the relation (7),

$$e(\Gamma, \mathfrak{U}) \geq 0. \quad (9)$$

(9°) *Definition.* Whether $d(\Gamma, \mathfrak{U})$ is finite or infinite,

$$\bar{e}(\Gamma, \mathfrak{U}) = \max \lim_{m \rightarrow \infty} e(\Gamma_m, \mathfrak{U}_m), \quad (10)$$

where Γ_m tends to Γ and \mathfrak{U}_m to \mathfrak{U} in such a way that $d(\Gamma_m, \mathfrak{U}_m)$ is finite for $m = 1, 2, 3, \dots$. Sequences of this kind always exist for any given (Γ, \mathfrak{U}) , since we may take \mathfrak{U}_m to have always a finite number of boundaries and finite genus, and Γ_m to consist of a finite number of polygons. The maximum in (10) is with respect to all such sequences. " $\overline{\lim}$ " denotes the superior limit.

Obviously, by (9), we have in every case,

$$\bar{e}(\Gamma, \mathfrak{U}) \geq 0. \quad (11)$$

(10°) *Definition.*

$$a(\Gamma, \mathfrak{U}) = \min \mathfrak{A}(S) \quad (12)$$

where $\mathfrak{A}(S)$ denotes the area of S , which ranges over all surfaces of topological type \mathfrak{U} bounded by Γ .

(11°) *Definition.*

$$a(\Gamma, \mathfrak{U}') = \min \mathfrak{A}(S') \quad (13)$$

where the range of S' consists of all surfaces of type \mathfrak{U}' bounded by Γ .

(12°) *THEOREM.*

$$a(\Gamma, \mathfrak{U}) = d(\Gamma, \mathfrak{U}), \quad \text{also } a(\Gamma, \mathfrak{U}') = d(\Gamma, \mathfrak{U}'). \quad (14)$$

4. We are now in a position to state our main theorems.

THEOREM 1. *If $d(\Gamma, \mathfrak{U})$ is finite, and we have the strict form of inequality³*

$$d(\Gamma, \mathfrak{U}) < d(\Gamma, \mathfrak{U}'), \quad (15)$$

then a minimal surface M exists, of topological type T , bounded by Γ .

The area of M is the least possible for its prescribed topological type and boundaries:

$$\mathfrak{A}(M) = a(\Gamma, \mathfrak{U}). \quad (16)$$

THEOREM 2. *Whether $d(\Gamma, T)$ is finite or infinite, if $\bar{e}(\Gamma, T)$ —always non-negative⁴—is actually positive:*

$$\bar{e}(\Gamma, \mathfrak{U}) > 0, \quad (17)$$

then a minimal surface M exists, of topological type \mathfrak{U} , bounded by Γ .

If $d(\Gamma, \mathfrak{U}) = a(\Gamma, \mathfrak{U})$ is infinite, then so is the area of M , but every completely interior sub-region M_1 of M has an area which is finite and a minimum for its own topological type \mathfrak{U}_1 and boundaries Γ_1 .

5. The proof of these theorems is practically the same as for the finite or algebraic case of Problem P_0 , requiring hardly more than the appropriate changes of wording. The same basic formulas (14), (15), (16) of Note II, are applied.

The important observation is that *the Variational Theorem of Note II (art. 7) continues to hold, mutatis mutandis, for all real analytic as well as algebraic curves \mathfrak{A} .*

We employ non-homogeneous line coördinates, namely the coefficients u, v in

$$y = ux + v. \quad (18)$$

We also take the point O in the statement of the Variational Theorem for origin, so that its line equation is $v = 0$. Then the line equation of the real analytic curve \mathbf{A} will have the form

$$(au^2 + bu + c)K(u) + vL(u, v) = 0, \quad (19)$$

where K, L are now *any analytic functions*, instead of polynomials, as in Note II. The quadratic factor

$$au^2 + bu + c = 0 \quad (20)$$

represents the two conjugate imaginary tangents t, \bar{t} . For the varied equation, we have

$$[(a + a'\epsilon)u^2 + (b + b'\epsilon)u + (c + c'\epsilon)]K(u) + vL(u, v) = 0, \quad (21)$$

quite analogous to Note II.

6. The detailed version of the present note will be published elsewhere under the same title.

¹ In accordance with certain two simple postulates, which express essentially that the angle-metric induced on R by this conformal representation of its neighborhoods on a circle is self-consistent whenever two neighborhoods overlap. Cf. H. Weyl, *Die Idee der Riemannschen Fläche*, 36 (1913); L. Ahlfors, "Geometrie der Riemannschen Flächen," *Proc. Oslo Congress*, 1, 239–248 (1936); S. Stoilow, "Sur la définition des surfaces de Riemann," *Ibid.*, 2, 143; S. Stoilow, *Principes topologiques des fonctions analytiques*, (1937).

² This does not contradict Weyl, loc. cit., p. 66: "*Jede Riemannsche Fläche ist zweiseitig.*" For this theorem is associated with the idea of an analytic function $f(t)$ on the Riemann surface, t being sharply distinguished from its conjugate \bar{t} , whereas our geometric form of definition obviously permits one-sidedness of R . In that case, we employ in our analysis a covering surface R_1 , which is two-sided and in two-one correspondence with R ; cf. Weyl, loc. cit., last lines of p. 61.

³ See (7).

⁴ See (11).

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

September 15, 1938

Number 9

Copyright 1938 by The National Academy of Sciences

FACET NUMBER AND THE v^+ HORMONE IN THE BAR EYE OF
*DROSOPHILA MELANOGASTER*¹

BY SIMON CHEVAIS, BORIS EPHRUSSI AND ARTHUR G. STEINBERG²

INSTITUT DE BIOLOGIE PHYSICO-CHIMIQUE, PARIS, FRANCE, AND COLUMBIA UNIVERSITY,
NEW YORK

Communicated August 4, 1938

Introduction.—Beadle and Ephrussi³ and Steinberg and Abramowitz⁹ have shown that eye discs of the Bar (*B*) "mutant" of *Drosophila melanogaster* when implanted, shortly before puparium formation, into vermillion (*v*) hosts are not autonomous with respect to pigmentation. It has been concluded therefore that the Bar eye normally receives some v^+ hormone^{3,10} from the body and that, at the time of transplantation, it either had not received the substance or having received it was not able to use it. In both the above cited papers evidence was offered which indicated that the reduced ability of the *B* eye to form the v^+ hormone was not causally related to its smaller size. In other words the effect of the *B* "locus" on the facet number and on the pigment are believed to result from two separate reactions, both of which may, however, have had a common origin. If this hypothesis is correct it should be possible to affect the facet number without affecting the eye color and *vice versa*.

Ephrussi, Khouvine and Chevais⁶ have shown that nitrogenous extracts of *Calliphora* larvae⁷ which contain the v^+ hormone also have the ability to increase the facet number when supplied to *B* larvae by the feeding technique of Beadle and Law.⁴ Provided the eye disc does not use the v^+ hormone prior to puparium formation (to be demonstrated below) the finding of Ephrussi, Khouvine and Chevais affords us a direct means of testing the above hypothesis.

Experiments and Discussion.—Two different experiments were performed to test the action of the *Calliphora* extracts on pigmentation before puparium formation: (a) *v B* larvae were raised on a medium consisting of a 2% agar solution containing 3% glucose and 11% of a nitrogenous extract of *Calliphora* larvae.⁷ Shortly before pupation the eye discs of these larvae were implanted into *v* hosts raised on the standard food. After eclosion the flies were dissected and the implanted eyes compared with *v B*

eyes taken from larvae of the same age which had been raised on the standard food and which had also been implanted into *v* hosts; (b) the same experiment as above was performed with flies which were vermilion not-Bar. In the latter case both the test and control eyes were of the same size, while in the former the test eyes were larger than the controls. In neither case, however, was any difference observed between the pigmentation of the experimental and control eyes. It is evident therefore that the *v*⁺ hormone supplied by the *Calliphora* extract does not produce a visible effect on the eye pigment under the conditions of the experiments reported in this paper. The method is therefore valid to test the independence of the ability to form the *v*⁺ hormone from the facet number.

Approximately forty *B* and forty *v B* larvae, 40 hours after hatching, were placed in vials containing 3 cc. of the agar-extract medium (these larvae will be referred to hereafter as "treated larvae"). The 40-hour stage was chosen because the extract has its maximum effect when larvae are grown on the standard food up to this age and then transferred to the extract (Chevais, unpublished). All the other larvae involved in these experiments were raised on the standard food medium. For the transplantation mature larvae were used as both hosts and donors. The experiments were performed at $25 \pm 0.2^\circ\text{C}$.

Eye discs from treated *B* males were implanted into *v* hosts. Males were used because the extract has a greater effect on them than on females.⁵ As controls for the degree of pigmentation, discs from treated *B* and *v B* larvae were implanted into wild type and *v* hosts, respectively; in addition *B* discs from untreated larvae were implanted into *v* hosts in order to have a direct comparison between them and treated *B*. Finally, as still further controls for the pigmentation, *B* and *v B* eye discs from untreated larvae were implanted into wild type and *v* hosts, respectively. The facet numbers of the implants recovered from the experiments involving the implantation of treated *B* into *v* were determined and compared with those of *B* implants from untreated larvae (unfortunately, the sex of the donors was not determined in these controls and hence no real mean value can be given; in the *B* stock employed in this experiment the females have approximately 15 fewer facets than the males⁶). The facets of the male sibs of both the treated and untreated *B* larvae were also counted. The facet counts, summarized in table 1, show clearly that there has been a marked increase in the number of facets in both the implanted and unimplanted (*in situ*) eyes developing from the treated larvae as compared with the untreated controls. Moreover, the range as well as the mean of the facet number of the implanted eyes taken from treated males is the same as that of the untransplanted sib males. In general the same may be said for the untreated controls. We may conclude therefore that transplantation does not affect the facet number. This of course is not at all unexpected since at the time

when the discs were taken for transplantation the facet number is already determined.⁸

The comparison of the pigmentation showed that the *B* eyes from the treated larvae implanted into *v* hosts were slightly darker than the *v B* controls⁹ (discs from treated *v B* larvae implanted into *v* hosts) and were identical in pigmentation with *B* eyes from untreated larvae which also had been implanted into *v* hosts.

The facet number of the treated *B* implants in *v* varies from 200 to more than 370 as shown in table 1; that is from the size of heterozygous double-infrabar (*B¹B¹/+*) to that of heterozygous Bar (*B/+*). Both *B¹B¹/+* and *B/+* when implanted into *v* hosts are very much darker than *v³,⁹*; consequently there was a sufficient increase in facet number to enable us to detect a change in the degree of pigmentation if there was a direct correlation between facet number and the ability to form the *v⁺* hormone.

TABLE 1
FACET NUMBERS OF BAR EYES RECOVERED UNDER THE VARIOUS EXPERIMENTAL CONDITIONS LISTED IN THE FIRST COLUMN

	M	RANGE	N
Eyes from treated ♂♂ implanted into <i>v</i> hosts	256.5	200-370*	8
<i>In situ</i> eyes from treated ♂♂	241.8	210-285	16
Implanted eyes from untreated ♂♂ and ♀♀	65.1†	52-86	7
<i>In situ</i> eyes from untreated ♂♂	85.3	68-109	15

* The largest eye was partially destroyed during the dissection; the true number is therefore higher than 370.

† See text.

Since no change in the degree of pigmentation was observed, the data may be accepted as a further verification of the original conclusion of Beadle and Ephrussi that the decreased ability of the Bar eye to form the *v⁺* hormone is not due to its smaller size.

Summary.—Eye discs from Bar larvae, which had been fed a nitrogenous extract of *Calliphora* larvae, known to increase the facet number, were implanted into vermilion hosts.

Comparison with the proper controls showed that such increased Bar eyes (up to 370 facets) develop the same pigmentation as do eyes from Bar larvae raised on the standard food medium.

It is concluded that there is no direct correlation between facet number and the ability of the eye to form the *v⁺* hormone.

These results are in agreement with the similar conclusion previously drawn by Beadle and Ephrussi.

¹ Work done at the Institut de Biologie physico-chimique, Paris, France.

² Recipient of a grant from the Dykman Fund of the Department of Zoölogy of Columbia University.

- ² Beadle, G. W., and Ephrussi, Boris, *Genetics*, **21**, 225-247 (1936).
 - ⁴ Beadle, G. W., and Law, L. W., *Proc. Soc. Exptl. Biol. Med.*, **37**, 621 (1938).
 - ⁵ Chevais, Simon, and Steinberg, Arthur G. (in press).
 - ⁶ Khouvine, Yvonne, Ephrussi, Boris, and Chevais, Simon, *Nature*, **141**, 204-205 (1938).
 - ⁷ Khouvine, Yvonne, and Ephrussi, Boris, *C. R. Soc. Biol.*, **124**, 885 (1937).
 - ⁸ Medvedev, N. N., *Zh. Ind. Abstam. Vererbl.*, **70**, 55-72 (1935).
 - ⁹ Steinberg, Arthur G., and Abramowitz, Mortimer, *Proc. Nat. Acad. Sci.*, **24**, 107-111 (1938).
 - ¹⁰ The v^+ hormone—a substance necessary for the formation of the wild type eye pigmentation; when supplied to v flies this substance causes the eye color to approach that of wild type.
- For the purposes of this experiment, v flies may be regarded as completely lacking the v^+ hormone.

AN X-RAY INDUCED INTERCALARY DUPLICATION IN *DROSOPHILA* INVOLVING UNION OF SISTER CHROMATIDS

BY B. P. KAUFMANN AND RUTH C. BATE

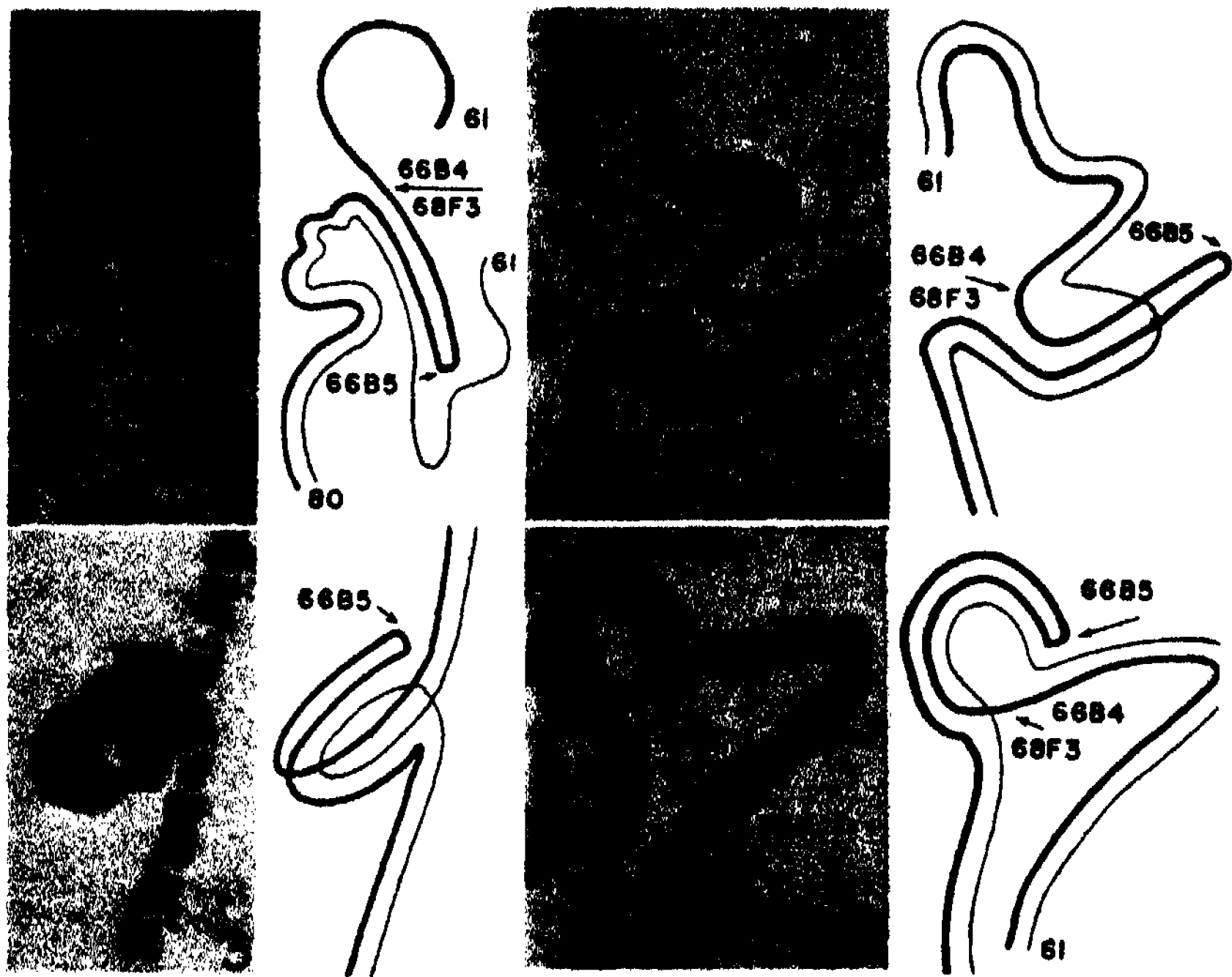
DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING
HARBOR, NEW YORK

Communicated August 13, 1938

In a study recently completed in this laboratory, Carlson¹ has found striking evidence that sister chromatids of broken sections of irradiated neuroblast chromosomes of the Orthopteran, *Chortophaga*, may fuse *inter se* at the breakage points to produce either chromatid bridges, ring-shaped or V-shaped fragments, depending on whether they originally occupied proximal, intercalary or distal positions. Bauer, Demerec and Kaufmann² in appraising the evidence for and against the possible occurrence of single induced breaks have suggested that the rarity of unequivocal cases of terminal deficiencies and terminal inversions in *Drosophila* may be due to their elimination rather than to non-occurrence. Assuming that two chromatids of the same chromosome could unite at a breakage point, a bridge would be formed as the spindle attachment regions separate at anaphase. Rupture across the bridge with subsequent refusion of chromatids, and repetition of the process in successive mitoses would increase the genic unbalance in daughter nuclei so that non-viable combinations would result.

The present report is presented to show that two chromatids of a chromosome of *Drosophila melanogaster* with breaks at identical loci may unite with one another at these broken ends, and that under suitable conditions the resulting rearrangement may be perpetuated.

Salivary glands secured from an F_1 female larval descendant of a father irradiated at 3000 r-units showed a duplicated section of the left limb of the third chromosome extending from 66B5 to 68F3 of Bridges' 1935 map. The duplication is arranged in the pattern of a "reversed repeat," as may be represented by the sequence *abcdgfeefghijk*. In several of the nuclei the two strands of paternal origin and the maternal chromosome had



FIGURES 1-4

Salivary gland chromosomes of intercalary duplication. In the diagrams shown to the right of each photograph the altered paternal strand is represented by the heavier line; the maternal, unaltered strand by the lighter line. The tip of 3L is marked by the figure 61; the spindle attachment end by the figure 80. Arrows indicate the inversion point and the limits of the duplicated section. Further description in the text.

conjugated to form a looped aggregate resembling the pairing configuration of an inversion heterozygote (figure 3). A striking feature of conjugation is the close association maintained between the duplicated regions of the 3L strand of paternal origin even when the maternal strand remains wholly or partially unpaired. As a result of this intimate lateral association the two linearly abutting homologous bands (the 66B5 bands) most frequently present the aspect of free ends of homologous strands (see figures 1, 2). A similar type of pairing of the duplicated intercalary sec-

tions inserted in the fourth chromosome in eyeless-dominant was observed by Bridges.³

Failure to observe evidence of linear continuity of the 66*B*5 bands suggested that we might possibly be dealing with a rearrangement involving translocation between the 66*B*5 ends and some poorly defined portion of the chromocentral region, such as those proximal chromomeres representing the short arms of the *X* and fourth chromosomes (Kaufmann⁴). Since the glands were obtained from an *F*₁ larva, it was impossible to secure additional salivary gland nuclei for study. Fortunately we had preserved the larval ganglia and were able to determine, as Bridges did in the case of eyeless-dominant, that the altered chromosome existed as a separate and continuous unit. Analysis of several mitotic figures of neuroblast and ganglion cells disclosed no perceptible alteration in the second, fourth or *X* chromosomes, although there was some intimation of difference in length between the two third chromosomes. We feel certain, therefore, that the sequence of banding in the altered third chromosomes is as follows:

61,62 . . . 66*B*4/68*F*3 . . . 66*B*5/66*B*5 . . . 68*F*3,69*A*1 . . . 80 sp. a. 81 . . 100

The apparent lack of linear continuity across the 66*B*5/66*B*5 union in the salivary chromosomes presumably represents a rupture at this point due to the force of lateral attraction between identical loci of the duplicated regions, causing the chromosome to fold through 180 degrees. Lack of such intimate pairing in most of the reversed repeats which are present in nature in the chromosomes of *Drosophila* may be the result of the establishment of mutational differences between the duplicated sections in the period intervening since their origin.

It seems also significant that the duplicated sections of paternal origin are paired in practically every nucleus, whereas these two conjugate with the maternal strand in only some of the cells. Such observations suggest that when pairing is initiated, the proximity of adjacent bands favors their synapsis and the consequent conjugation of the duplicated sections prior to their union with the strand of maternal origin.

Whether the duplication results from strands which were split at the time of irradiation, or whether division and reunion occurred subsequently cannot be determined from this rearrangement. Data bearing on these problems have been obtained from other duplications and will be discussed in another publication.⁵ In the present case we may assume that breaks occurred in 66*B* and in 68*F*, that at the time of reunion of the fragments the region between these points was present as twin strands, that the 68*F* end of one of the strands joined with the 66*B*4 end of the distal section of 3*L* to give an inverted section, and that the sister chromatids then fused at the 66*B*5 broken ends. The 68*F*3, 69*A*1 continuity may represent an unaltered sequence resulting from the fact that at the time of irradiation the chromosome had divided at this level and that only one

strand was broken, or the 68*F*3, 69*A*1 sequence may represent a refusion which would be necessary if both strands had broken at this level. The general plan of the rearrangement may be expressed as follows:

$$\begin{array}{l} abcd|efg|hijk \cdot lmnopqrstu \\ abcd|efghijk \cdot lmnopqrstu \end{array} \quad \text{giving } abcdgfeefghijk \cdot lmnopqrstu$$

with probably another strand of the constitution: $abcdhijk \cdot lmnopqrstu$. The fate of the latter chromosome remains conjectural, although it may as a result of the deficiency form a non-viable nucleus at the end of the first cleavage mitosis.

The union of sister chromatids of a chromosome in the manner here described offers a possible explanation of the origin in nature of the reversed repeats described by Bridges^{6,7} and shown especially clearly in the 35*F*–36*D* regions of the left limb of the second chromosome. The same type of rearrangement has been postulated by Offerman⁸ as an explanation of his interpretation of the structure of the bulb in division 2 of the *X* chromosome as a reversed repeat. If in such rearrangements the region of mirror-imaging is not too extensive to affect viability, and if it has selection value in nature, it may be expected to be perpetuated in phylogeny.

Summary.—An intercalary mirror-imaged duplication in the left limb of the third chromosome of *D. melanogaster* was found in an *F*₁ larval descendant of an irradiated father. Origin of the duplication is attributed to fusion at identical loci of the broken ends of two chromatids of the paternal third chromosome.

¹ Carlson, J. G., *Genetics* (in press).

² Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics* (in press).

³ Bridges, C. B., *Trans. Dynamics of Develop.*, **10**, 463–474 (1935).

⁴ Kaufmann, B. P., *Jour. Morph.*, **56**, 125–155 (1934).

⁵ Kaufmann, B. P. (in preparation).

⁶ Bridges, C. B., *Jour. Hered.*, **26**, 60–64 (1935).

⁷ Bridges, C. B., *Ibid.*, **29**, 11–13 (1938).

⁸ Offerman, C. A., *Jour. Genetics*, **32**, 103–116 (1936).

THE DISTRIBUTION OF GENE FREQUENCIES IN POPULATIONS OF POLYPLOIDS

BY SEWALL WRIGHT

DEPARTMENT OF ZOÖLOGY, THE UNIVERSITY OF CHICAGO

Communicated August 12, 1938

The theoretical distribution of gene frequencies in populations of diploid organisms, as affected by mutation, selection, migration and inbreeding, has been discussed in previous papers.^{1,2} It is desirable to extend the theory to populations of polyploids.

Mutation and Migration.—Assume gene frequencies $[(1 - q)A + q a]$ where a is the gene under consideration and A represents its array of alleles (without any implications with respect to dominance). Zygotic frequencies rapidly approach an equilibrium under random mating, the equilibrium distribution in $2k$ -ploids being the expansion of $[(1 - q)A + q a]^{2k}$ (Haldane³).

Mutation pressure is the same as with diploids. Letting u be the rate of mutation from a , and v the rate of mutation to a , the rate of change of gene frequency per generation is

$$\Delta q = v(1 - q) - uq. \quad (1)$$

Crossbreeding pressure also is the same as with diploids. Letting q_i be the gene frequency of the species as a whole and m the effective amount of exchange between the local population in question and the species as a whole we have:

$$\Delta q = -m(q - q_i). \quad (2)$$

Any results for mutation pressure can be transformed into ones for crossbreeding pressure by substituting $m(1 - q_i)$ for u and mq_i for v . For simplicity, we will develop conclusions for mutation pressure only.

Selection Pressure.—The simplest kind of selection is that in which the selective values (W) of the various zygotes deviate from standard in proportion to the number of replacements of type genes (the case of no dominance).

ZYGOTE	FREQUENCY	W
A^{2k}	$(1 - q)^{2k}$	1
$A^{2k-1}a$	$2kq(1 - q)^{2k-1}$	$1 - s$
.....
a^{2k}	q^{2k}	$1 - 2ks$
	$\bar{W} = 1 - 2ksq.$	(3)

$$\Delta q = - \frac{sq(1-q)}{1-2ksq} = \frac{q(1-q)d\bar{W}}{2k\bar{W}dq} \quad (4)$$

In the more general case in which \bar{W} is not related linearly to q because of more or less dominance, the momentary selective advantage of replacing A by a is still $\frac{d\bar{W}}{dq}$ and the formula for Δq is still given by (4).

Still more generally, if there is interaction between different series of alleles, and a selective value, W , is assigned each combination, the rate of change of the frequency of a particular gene under selection (with specified values of all other gene frequencies) is given by the formula

$$\Delta q = - \frac{q(1-q)}{2k\bar{W}} \frac{\partial \bar{W}}{\partial q} \quad (5)$$

Inbreeding.—The effect of restriction in the effective size of population can be worked out by means of path coefficients.^{1,4,5} In a $2k$ -ploid the zygote may be considered as equally and completely determined by the $2k$ component genes.

Let y be the path coefficient measuring the contribution of gene to zygote and let f be the correlation between genes due to inbreeding. Expressing the complete determination of zygote by genes in an equation:

$$2ky[y + (2k-1)yf] = 1.$$

$$y^2 = \frac{1}{2k[1 + (2k-1)f]} \quad (6)$$

The path coefficient (x) expressing the contribution of the zygote to a single gene of a gamete must equal the correlation coefficient as there is only one connecting path.

$$x = y + (2k-1)yf$$

$$x^2 = \frac{1 + (2k-1)f}{2k} \quad (7)$$

$$xy = \frac{1}{2k} \quad (8)$$

The zygote may also be considered as equally and completely determined by the two uniting gametes and each of these by its component genes. From this point of view, there are two kinds of correlation between component genes: between genes of the same gamete (c) and between genes of different gametes (d). The average correlation (f) is thus:

$$f = \frac{(k-1)c + kd}{2k-1} \quad (9)$$

Under the simplest theory of segregation in polyploids (Muller⁶) the correlation between genes of the same gamete must be the same as between two *different* genes of the parental zygote. Using primes to designate preceding generations

$$c = f'. \quad (10)$$

There is a possibility (greatest for loci remote from the spindle fibre) that a gamete in a polyploid may contain more than one representative of the same parental gene (Haldane³) but the possible modification of the results due to this complication cannot be great and will not be considered here.

The correlation between genes of uniting gametes can be obtained by tracing the connecting paths according to the system of mating.

The proportion of unlike pairs of genes among pairs drawn from zygotes will be represented by p . In diploids this is the percentage of heterozygosis.¹ The relation of p to f is easily found by constructing the correlation table for f . For the present purpose two A 's are considered like genes (two non- a 's) even though they may actually be different alleles of a .

	A	a	
a	$p/2$	$q - p/2$	q
A	$1 - q - p/2$	$p/2$	$1 - q$
	$1 - q$	q	1

$$f = 1 - \frac{p}{2q(1 - q)}. \quad (11)$$

Self-Fertilization.—In this case $d = x'^2$. From this result and (9), (10) and (7)

$$f = \frac{1 + (4k - 3)f'}{2(2k - 1)}. \quad (12)$$

$$p = \left[\frac{4k - 3}{4k - 2} \right] p'. \quad (13)$$

Following are special cases:

Diploid	$p = \frac{1}{2}p'$	Hexaploid	$p = \frac{9}{10}p'$
Tetraploid	$p = \frac{5}{6}p'$	Octoploid	$p = \frac{13}{14}p'$

Haldane³ has studied the effects of self-fertilization by a direct consideration of all types of zygotes. He represents the symmetrical case in tetraploids as $p_n A^4 : q_n A^3 a : r_n A^2 a^2 : q_n A a^3 : p_n a^4$ where $2p_n + 2q_n + r_n = 1$, $p_1 = q_1 = 0$, $r_1 = 1$.

He shows that the following relations hold

$$\begin{aligned}p_{n+1} &= p_n + \frac{1}{4}q_n + \frac{1}{36}r_n \\q_{n+1} &= \frac{1}{2}q_n + \frac{2}{9}r_n \\r_{n+1} &= \frac{1}{2}q_n + \frac{1}{2}r_n.\end{aligned}$$

The proportion of unlike pairs of genes in zygotes (p of the present paper) is $q + (2/3)r$. It may easily be seen that $(q_{n+1} + 2/3r_{n+1}) = 5/6(q_n + 2/3r_n)$ or $p = 5/6p'$ in our terminology.

Haldane has similarly derived the consequences of self-fertilization in hexaploids. Our formula, $p = 9/10p'$ is in agreement.

GROUPS OF N MONOECIOUS INDIVIDUALS

Random Self-Fertilization.—Extension can easily be made to larger inbreeding groups. In the case of N monoecious individuals with self-fertilization at random the chance of selfing is $\frac{1}{N}$ and of union of gametes from different individuals is $\frac{N-1}{N}$. Giving due weight to these two possibilities

$$d = \frac{1}{N}x'^2 + \frac{N-1}{N}(4k^2x'^2y'^2)d' \quad (14)$$

$$= \frac{1}{2Nk} [1 + (2N-1)(2k-1)f' - (2N-2)(k-1)f'']$$

$$p = \frac{1}{2N(2k-1)} [(6Nk-4N-2k+1)p' - (2N-2)(k-1)p'']. \quad (15)$$

This reduces to $p = \left(\frac{4k-3}{4k-2}\right)p'$ if $N = 1$ (self-fertilization) but if N is large it is approximately $p = \left(1 - \frac{1}{2Nk}\right)p'$.

No Self-Fertilization.—With N monoecious individuals and self-fertilization excluded, the chance of matings between siblings is $\frac{2}{N(N-1)}$,

between half-siblings is $\frac{4(N-2)}{4(N-1)}$ and of more remote matings

$$\frac{(N-2)(N-3)}{N(N-1)}.$$

Using these weights:

$$d = \frac{1}{N(N-1)}(x'^2 + d') + \frac{N-2}{N(N-1)}(x'^2 + 3d') + \frac{(N-2)(N-3)}{N(N-1)}d'. \quad (16)$$

$$p = \frac{1}{2N(2k-1)} [(6Nk - 4N - 4k + 2)p' - (2Nk - 2N - 4k + 3)p'']. \quad (17)$$

This also reduces approximately to $p = \left(1 - \frac{1}{2Nk}\right) p'$ if N is large.

Mating of Siblings.—If $N = 2$ in the preceding case (mating of siblings)

$$p = p' - \frac{1}{8k-4} (2p' - p''). \quad (18)$$

With constant size of population it is legitimate to put $p/p' = p'/p''$ to find the limiting relation between successive generations.

$$p = \left[\frac{4k-3 + \sqrt{16k^2 - 16k + 5}}{8k-4} \right] p' \text{ approximately.} \quad (19)$$

Following are special cases:

	EXACT	LIMITING
Diploid	$p = p'/2 + p''/4$	$p = 1/4(1 + \sqrt{5})p' = 0.80902p'$
Tetraploid	$p = 5/6p' + p''/12$	$p = 1/12(5 + \sqrt{37})p' = 0.92356p'$
Hexaploid	$p = 9/10p' + p''/20$	$p = 1/20(9 + \sqrt{101})p' = 0.95249p'$
Octoploid	$p = 13/14p' + p''/28$	$p = 1/28(13 + \sqrt{197})p' = 0.96556p'$

Bartlett and Haldane⁷ gave the limiting result in tetraploids in a form equivalent to $p = 0.92356p'$, from the pertinent solution of an octic equation derived from the iteration equations for all possible types of mating. This agrees with our result.

Sampling Variance.—In a population of N $2k$ -ploids with gene frequencies $[(1-q)A + qa]$ and random association of genes in the gametes (i.e., $p = 2q(1-q)$) the variance of q among progeny populations, resulting from random samples of $2N$ gametes is

$$\sigma_{\Delta q}^2 = \frac{q(1-q)}{2Nk}. \quad (20)$$

Unfortunately the association of genes in gametes is not wholly a random one. For example, a tetraploid $AAaa$ produces gametes in the proportions $1/6 AA : 4/6 Aa : 1/6 aa$ (if A is near the spindle fibre) while under random association the proportions would be $1/4 AA + 2/4 Aa + 1/4 aa$. However, the departure from (20) is slight except in very small populations. In the extreme case of self-fertilization ($N = 1$) it may easily be shown that

$$\sigma_{\Delta q}^2 = \frac{q(1-q)}{4k-2}.$$

The Distribution of Gene Frequencies.—A formula for the distribution of gene frequencies in populations subject to evolutionary pressure Δq and sampling variance $\sigma_{\Delta q}^2$ was reached in a previous paper.⁵

$$\varphi(q) = (C/\sigma_{\Delta q}^2) e^{2\int (\Delta q/\sigma_{\Delta q}^2) dq} \quad (21)$$

The approximate formula for the distribution of q in $2k$ -ploids is thus as follows, letting $\Delta q = v(1 - q) - uq + \frac{q(1 - q)}{2k\bar{W}} \frac{d\bar{W}}{dq}$ from combination of (1) and (4) and letting $\sigma_{\Delta q}^2 = q(1 - q)/2Nk$.

$$\varphi(q) = C\bar{W}^{2n} q^{4nkv - 1} (1 - q)^{4nku - 1} \quad (22)$$

The joint distribution for a system of multiple genes can be written as follows, letting \bar{W} here be the mean selective value of the population in terms of all gene frequencies as variables.

$$\varphi(q_1 \dots q_n) = C\bar{W}^{2n} \prod [q_i^{4nkv_i - 1} (1 - q_i)^{4nku_i - 1}] \quad (23)$$

It is to be noted that this applies to aneuploids (k variable) as well as to euploids (k constant).

¹ Wright S., *Genetics*, 6, 111-178 (1921).

² Wright S., *Proc. Nat. Acad. Sci.*, 23, 307-320 (1937).

³ Haldane, J. B. S., *Jour. Genetics*, 22, 359-372 (1930).

⁴ Wright, S., *Proc. Nat. Acad. Sci.*, 19, 411-420 (1933).

⁵ Wright, S., *Ann. Math. Stat.*, 5, 161-215 (1934).

⁶ Muller, H. J., *Amer. Nat.*, 48, 508-512 (1914).

⁷ Bartlett, M. S., and J. B. S. Haldane, *Jour. Gen.*, 29, 175-180 (1934).

⁸ Wright, S., *Proc. Nat. Acad. Sci.*, 24, 253-259 (1938).

THE MECHANISM OF DELAYED KILLING OF MAIZE SEEDS WITH X-RADIATION

BY LOUIS R. MAXWELL

BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE

Communicated August 8, 1938

Biological response to x-rays has often been interpreted on the basis of the quantum theory of radiation absorption and the so-called hit theory¹ involving the concept of a particular sensitive volume within the material. For the death of single-celled individuals it is frequently found that survival-dosage curves show a simple exponential relationship indicating the presence of one sensitive volume which requires only one hit or penetration by an electron in order to produce death. When multi-cellular systems

are studied it is usually found that the survival curves are no longer exponential but have a sigmoid shape. The hit theory in this case has two indistinguishable alternatives; either death occurs from a number of hits within one sensitive volume or a different number of hits within two or more sensitive volumes.

Certain tests of this theory will be described for the x-ray treatment of corn seeds. In this instance the seed embryos under investigation are large enough for scanning experiments designed for the purpose of searching for the position of a possible single sensitive volume. Killing in this case comes at a particular stage in the growth of the seedlings as a result of irradiation of the dry seeds with dosages ranging from 50,000 to 100,000 "r" units. Within approximately one week after the emergence of the plumule elongation ceases and usually a thick crumpled first leaf will show. After elongation has stopped the seedling remains green for about another week before dying. The maximum height obtained is about 1 to 2 cm. This type of death has been called delayed killing² and figure 1 illustrates the manner in which it occurs. A continuous band of radiation is used with a maximum intensity at 0.50 Å and a short wave-length limit of 0.26 Å. Dosage values are given to within 5 per cent as determined by an open air ionization chamber.³

Experimental survival ratio-dosage relationships are shown in figure 2 where a sigmoid-shaped curve is obtained. Interpretation of these results can be made from a consideration of the hit theory which is formulated in the following manner:¹ Given Z_0 seeds each with one sensitive volume $v(\text{cm.}^3)$ which requires n successive hits or penetrations by primary electrons in order to produce delayed killing, then the number Z which survive a dosage of q ("r" units) will be given by the following Poisson series:

$$\frac{Z}{Z_0} = e^{-\alpha q} \left(1 + \alpha q + \frac{(\alpha q)^2}{2!} + \frac{(\alpha q)^3}{3!} + \dots + \frac{(\alpha q)^{n-1}}{(n-1)!} \right) \quad (1)$$

where α represents the number of times the sensitive volume v is hit by primary electrons per "r" unit measured. It has been shown by Glocker¹ that when the range of the primary electrons is taken into account

$$\alpha = N_0 \cdot \frac{R + a}{a} \cdot v \quad (2)$$

N_0 being the number of primary electrons formed per cm.^3 per "r" unit measured, R the range of the primary electrons in the biological material and a the average path traversed by the primary electrons in a spherical sensitive volume of radius r when $R \gg a$, thus $a = \frac{4}{3}r$. Equation 1 can be evaluated by means of the incomplete gamma-functions from tables

prepared by Karl Pearson. A theoretical survival curve which approximately fits the experimental curve is shown in figure 2. In this case α

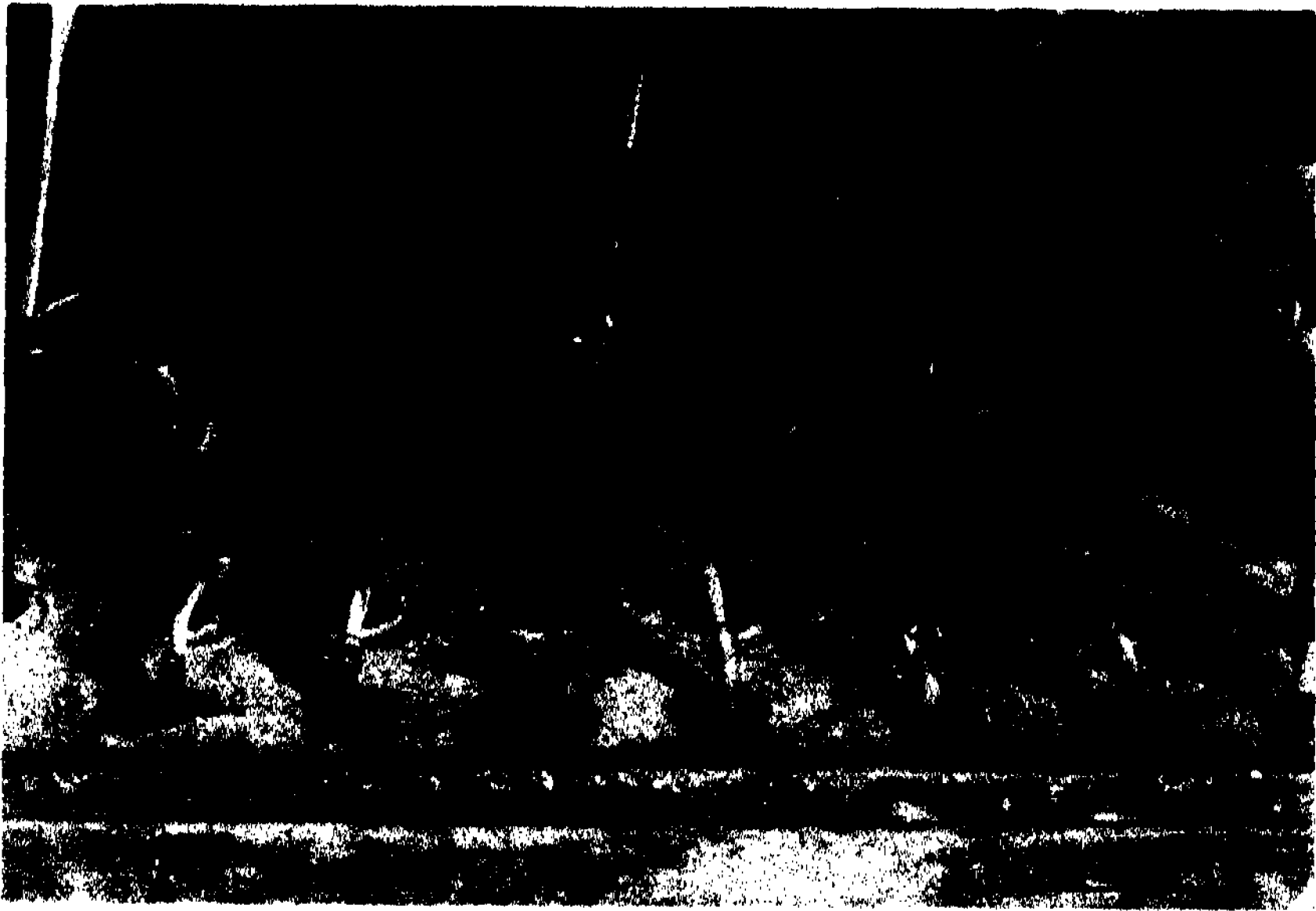


FIGURE 1

Photograph of delayed killed corn plants shown in the first row as compared with normal seedlings.

was taken equal to 4.5×10^{-4} ("r" units)⁻¹ which established the relationship between α and q . A value of $n = 14$ was obtained by selecting

TABLE 1
SUMMARY OF RESULTS OBTAINED WITH 1/2 MM. SLIT WIDTH

ZONE NUMBER IRRADIATED	DONAGE "r" UNITS	NUMBER OF SEEDS GERMINATED	PERCENTAGE SHOWING DELAYED KILLING	MEAN HEIGHT OF DRAD PLANTS (CM.)
6	50,000	83	none
7	50,000	45	none
8	50,000	125	none
9	50,000	42	none
10	50,000	274	none
11	50,000	42	none
12	50,000	123	none
13	50,000	42	none
14	50,000	122	none
16	50,000	81	none
10	100,000	60	none
10	300,000	248	43.0	6.6 ± 0.8*

* Standard error of mean

the proper slope for the theoretical curve. The spherical sensitive volume required by equation 2 is approximately 1×10^{-16} cm.³ assuming the primary electrons to be the effective particles. This calculation was made on the basis of monochromatic radiation of wave-length 0.50 Å which is close to the average wave-length used.

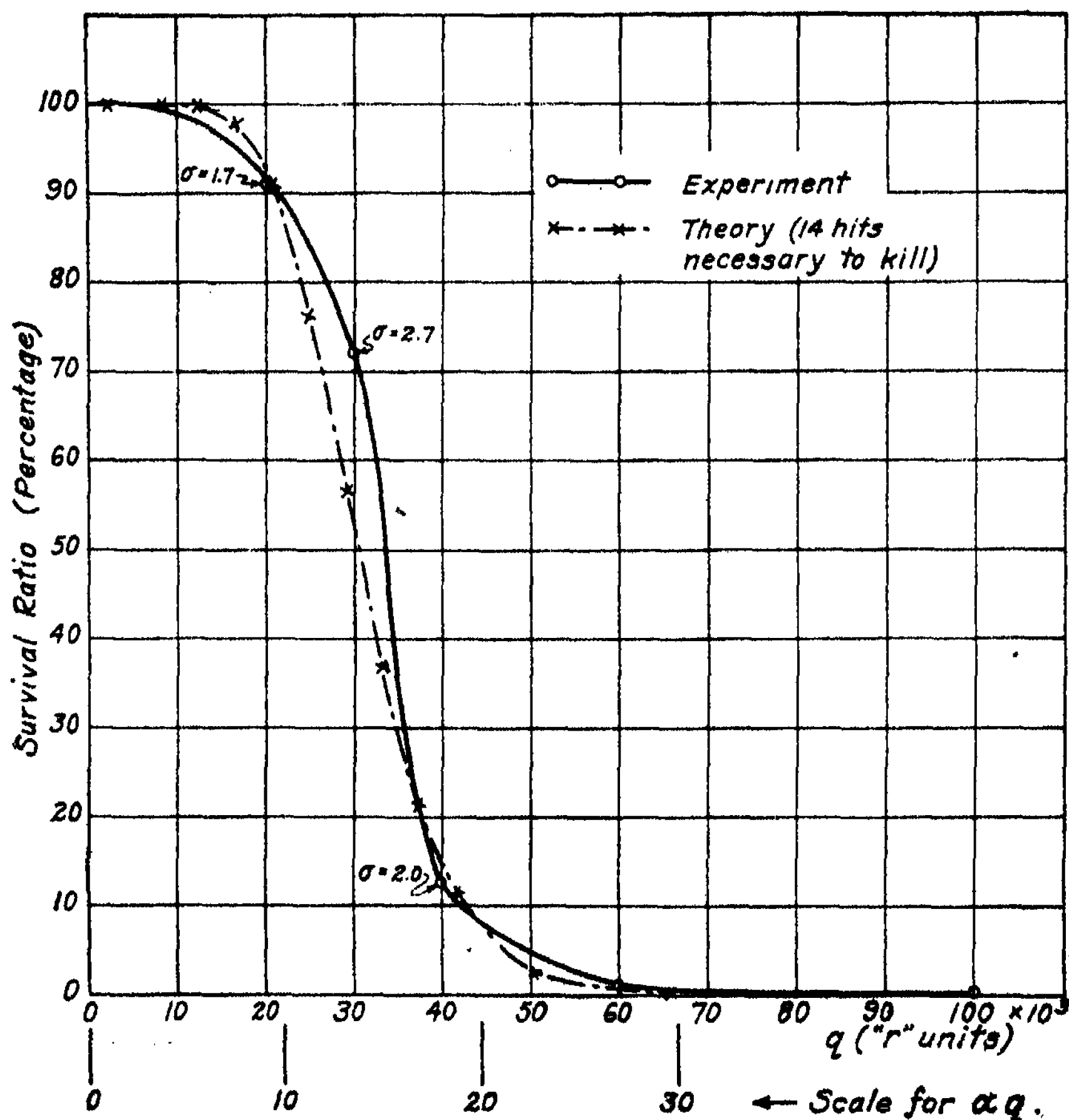


FIGURE 2

Survival ratio curves for the delayed killing of corn seeds. Values for the standard deviation (σ) are given for the experimental points.

A search for this sensitive volume was made by irradiating various assigned zones laid out crosswise of the seed as indicated in figure 3, while figure 4 shows the seed rack with lead slits used for the scanning experiments. Photographic films placed in the position occupied by the seed embryos provided a test for the actual width of the irradiated area ob-

tained. In table 1 are given the results of treatment taken over the length of the embryos with an irradiation area $\frac{1}{2}$ mm. in width. On the basis of a single sensitive volume contained within the embryo (which is about 7 mm. long) it is expected on the average that at least one out of every fourteen seeds or not less than 7% of the seeds should be killed. However, it is seen from table 1 that no delayed killing is observed for dosages of 100,000 "r" units or less involving treatment of more than 1000 seeds. The probability that this failure to kill could be the result of random sampling is so remote as to *eliminate the possibility of the existence of a single "sensitive volume" as defined by the hit theory.* At 300,000 "r" units a large

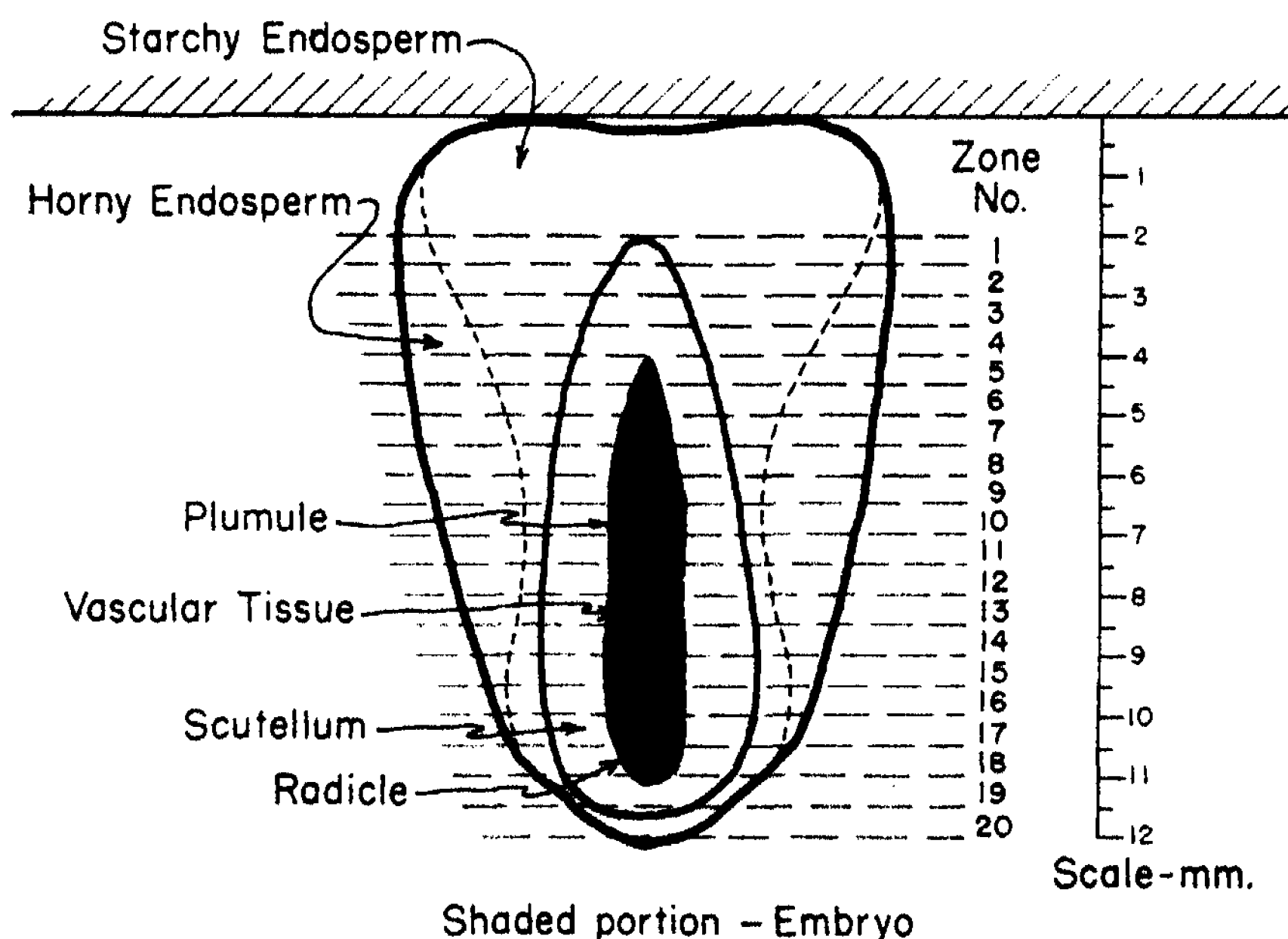


FIGURE 3

Drawing of a typical corn seed showing various zones used for irradiation.

number of the plants were killed but at a later stage in growth, indicating a different type of response.

Table 2 shows the results obtained for various wider slit widths and for exposures taken through the central portion of the seed embryos. It is seen that death occurs at the lower dosages and that, in general, a larger percentage of deaths will take place as the slit width is increased. However, in no case does complete killing exist even at the higher dosages. *The percentage death approaches a saturation value thus indicating that it is not proportional to the total amount of energy absorbed in a restricted region of the seed.* From table 2 it is also noticed that the plants die at earlier

stages as the slit width is increased, approaching for a 3 mm. slit width the average height of 1 to 2 cm. found for delayed death when the entire seed is exposed.

TABLE 2
SUMMARY OF RESULTS OBTAINED WITH 1.0 MM., 1.5 MM., 2.0 MM. AND 3.0 MM. SLIT WIDTHS

SLIT WIDTH	ZONE NUMBER IRRADIATED	DOSEAGE "r" UNITS	NUMBER OF SEEDS GERMINATED	PERCENTAGE SHOWING DELAYED KILLING	MEAN HEIGHT OF DEAD PLANTS (CM.)
1.0 mm.	9, 10	20,000	44	36.4	2.1
	9, 10	29,200	176	16.9
	9, 10	30,000	45	48.9	6.6
	9, 10	40,000	44	52.3	7.5
	9, 10	50,000	393	33.6
	9, 10	52,000	134	30.5
	9, 10	60,000	45	48.9	9.8
	9, 10	70,000	247	47.0	6.8
	9, 10	80,000	43	51.2	6.8
	9, 10	90,000	44	50.0	7.0
	9, 10	95,000	88	63.6	4.4
	9, 10	100,000	332	38.6	6.0
	9, 10	150,000	112	36.6	4.8
1.5 mm.	LH* 9, 10, 11, UH 12	31,600	177	10.7
	8, 9, 10	40,000	34	2.9	1.2
	LH 9, 10, 11, UH 12	43,500	32	69.0	3.8 \pm 0.9
	LH 9, 10, 11, UH 12	54,500	129	44.4
	8, 9, 10	80,000	71	49.5	3.4
	8, 9, 10	120,000	65	57.0	2.2
	8, 9, 10	160,000	36	50.0	1.2 \pm 0.5
	LH 9, 10, 11, UH 12	200,000	21	67.0	0.8 \pm 0.9
	LH 9, 10, 11, UH 12	300,000	18	56.0	3.0 \pm 1.2
2.0 mm.	9, 10, 11, 12	100,000	79	64.1	3.3 \pm 0.75
3.0 mm.	8, 9, 10, 11, 12, 13	20,000	36	none
	8, 9, 10, 11, 12, 13	33,200	178	23.6
	8, 9, 10, 11, 12, 13	40,000	69	50	2.2
	8, 9, 10, 11, 12, 13	58,000	132	70
	8, 9, 10, 11, 12, 13	60,000	69	72	1.7 \pm 0.28
	8, 9, 10, 11, 12, 13	100,000	33	87	1.4
	8, 9, 10, 11, 12, 13	150,000	30	87	2.0
	8, 9, 10, 11, 12, 13	300,000	21	95	1.6

* LH means lower half. UH means upper half.

Variations given represent standard error of mean.

Probing experiments taken over the entire length of the seed with a 2 mm. slit width are summarized in table 3. It is seen that there is a wide distribution of positions on the seed in which death occurs, with the most sensitive region located at the approximate center of the embryo. One

hundred seeds were dissected and measurements taken of the variation in position of the seed embryos. It was found that the position of the center of the embryo will have a standard deviation of approximately 0.5 mm. This fluctuation may account in part for the wide distribution of susceptible volumes. However, these sensitive volumes are many magnitudes larger than expected on the pure formal hit theory as described above.

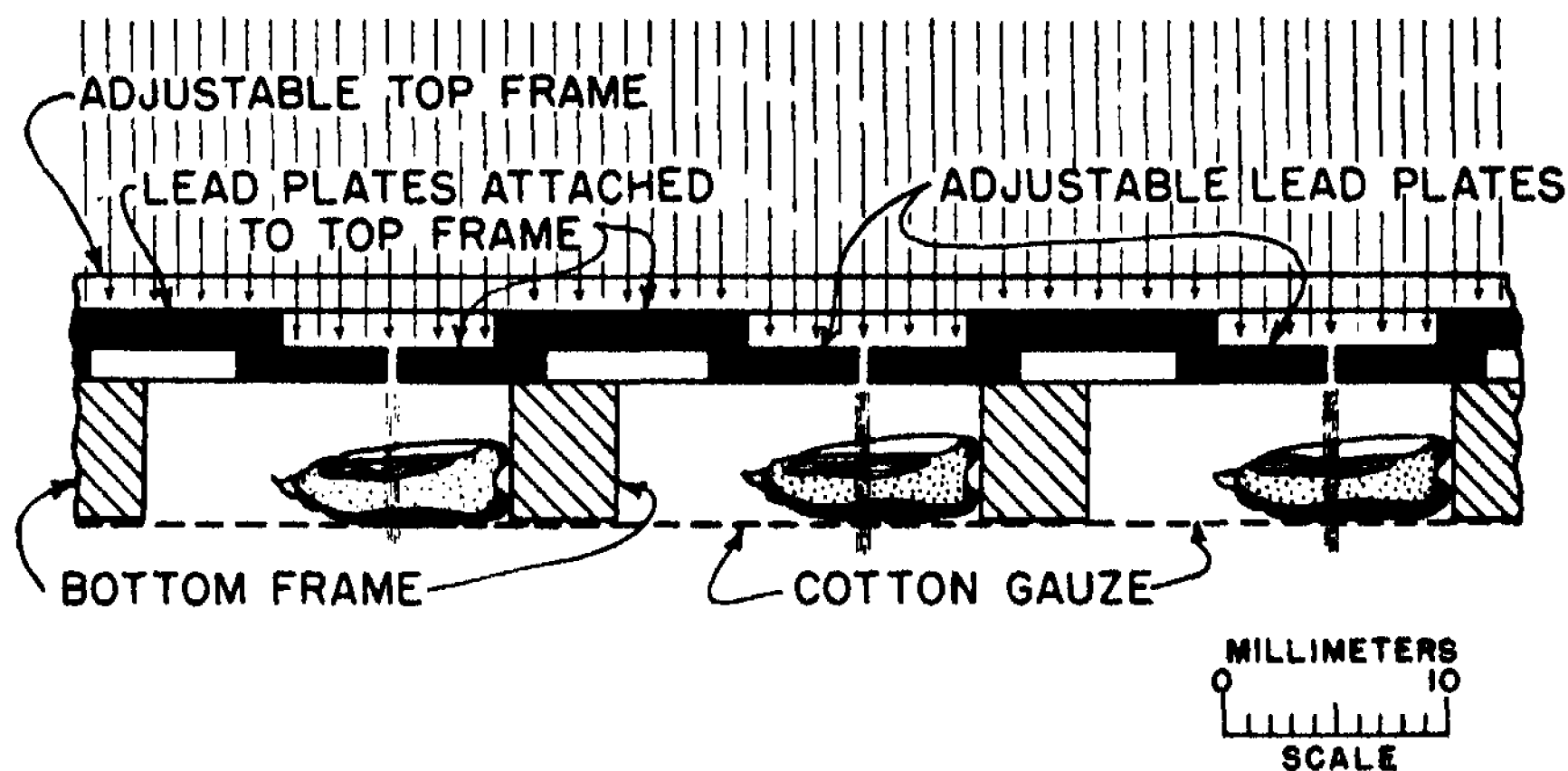


FIGURE 4

Diagram of lead slit arrangement used for irradiation.

For a multi-cellular organism such as a corn seed it is possibly not surprising that the hit theory interpretation based on the response of a single sensitive volume should fail. The alternative explanation involving a distribution of several sensitive volumes each requiring a certain number of

TABLE 3
RESULTS OBTAINED FROM SCANNING WITH 2.0 MM. SLIT WIDTH

ZONE NUMBER IRRADIATED	DOSAGE "r" UNITS	NUMBER OF SEEDS GERMINATED	PERCENTAGE SHOWING DELAYED KILLING	MEAN HEIGHT OF DEAD PLANTS (CM.)
1, 2, 3, 4	100,000	78	1.3	9.5
5, 6, 7, 8	100,000	82	32.1	2.1 \pm 0.35
9, 10, 11, 12	100,000	79	64.1	3.35 \pm 0.75
13, 14, 15, 16	100,000	81	11.1	6.51 \pm 1.10
17, 18, 19, 20	100,000	76	0.0

Variations given represent standard error of mean.

hits is not eliminated. However, on the basis of the above experiments it is safe to conclude that if multiple sensitive volumes do exist they are not contained within a spherical volume whose diameter is less than 0.5 mm.

The writer is indebted to Mr. G. N. Collins and Mr. J. H. Kempton, Bureau of Plant Industry, U. S. Department of Agriculture, for their helpful coöperation. They have made this work possible by taking charge of the planting and the collection of the data used on the growth of the plants.

¹ See for instance Glocker, R., *Zeit. Phys.*, **77**, 653-675 (1932).

² Collins, G. N., and Maxwell, L. R., *Science*, **83**, 375-376 (1936); Maxwell, L. R., *Phys. Rev.*, **51**, 375 (1937).

³ For the design used see Taylor, L. S., and Singer, G., *Radiology*, **15**, 637-646 (1930).

CONTACT EFFECTS BETWEEN PLANT ROOTS AND SOIL COLLOIDS

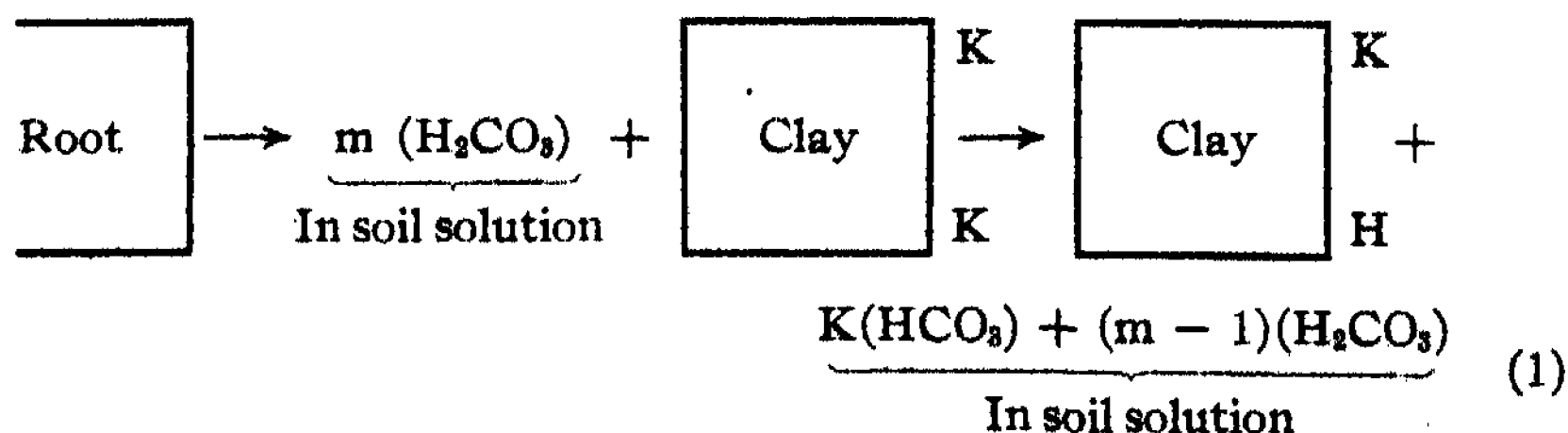
BY H. JENNY AND R. OVERSTREET

DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA

Communicated August 1, 1938

Introduction.—The prevailing theories of mineral absorption by plants from soils are based on the concept of the *soil solution*. They postulate that a nutrient element must be an integral part of the soil solution before it can be taken up by plant roots. An ion is said to be in the soil solution when it is detached from the solid phase and can diffuse freely. It closely follows the movements of the liquid phase. Essentially, the soil solution is identified with the nutrient solution of the plant physiologist.

Equation (1) schematically illustrates the action of plant roots in soils as visualized by the soil solution theory.



The roots excrete carbonic acid into the liquid phase surrounding the soil particles. The H ions replace K from the surface of soil colloids and the resulting potassium bicarbonate is now ready for intake by roots.

In this paper an additional mechanism of mineral intake by plants from soils is proposed. It is based on the phenomenon of ion interchange existing between two surfaces which are in contact.

Theory of Contact Exchange.—Colloidal clays are negatively charged and possess high capacities for cation adsorption. The cations on the surface of the colloidal particles are not held rigidly. As a result of thermal agitation the ions oscillate and, at times, may be at considerable distances from the surface; but they remain in the field of force emanating from the colloid. Although the ions are surrounded by water molecules, they are not in solution in the sense that they can diffuse freely. The cations are under conditions of constraint and follow closely the movements of the colloidal particle to which they are attached.

These surface cations may be freed by exchange and then become an integral part of the intermicellar liquid or soil solution. According to the kinetic theory of ionic exchange¹ release may occur when a replacing ion enters the oscillation space of the adsorbed cation, as illustrated in figure 1. This process forms the basis for the mechanism represented by equation (1).

Conceivably, ionic exchange might also occur if two oscillation spaces overlap. This process may take place between neighboring ions on the



FIGURE 1

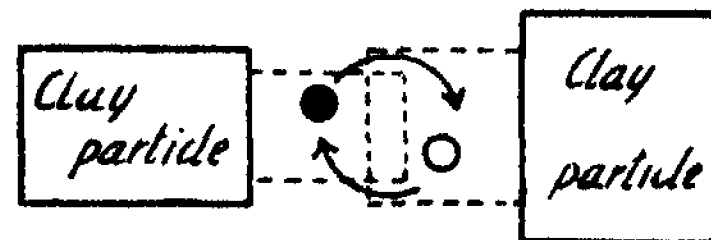


FIGURE 2

same surface and gives rise to the phenomenon of surface migration of ions. On the other hand if two separate colloidal particles approach so closely that the oscillation spaces interpenetrate, conditions for *contact exchange* between the two different particles exist as shown in figure 2. In this case the reaction as such is independent of the nature of the intermicellar liquid or soil solution. To use a picturesque expression one might say that the ions do not enter the soil solution *per se* but, in the moment of contact, jump directly from one particle to another.

The above considerations refer to ion transfer between particles of equal sign of any colloidal system. If roots with negative surfaces are immersed in colloidal clay suspensions (negative particles) there is good reason to believe that contact exchange might enter into play. Nutrient cations adsorbed on clay particles could thus become attached to plant roots directly, rather than by way of the soil solution. Since contact exchange involves a mutual transfer of ions, it follows that for every cation gained by the root an equivalent number of ions must leave the root surface and be transferred to the clay. For this reason we must distinguish between *contact intake* and *contact depletion*, as depicted in figures 3 and 4.

The experiments presented in this paper were designed to search for the

existence of contact phenomena between plant roots and colloidal clay particles. All efforts were concentrated on the phenomenon of contact depletion which is a particularly striking one, since the soil solution theories do not operate with the concept of depletion of roots. In this first paper data on the element potassium only will be reported.

Experimental Technique.—All experiments were conducted with barley plants of the Sacramento variety. They were grown according to the method of Hoagland and Broyer² and were used when they were three weeks old and about 18 inches high. The following types of systems were investigated.

(a) *Excised Roots.* About 100 grams of fresh roots were immersed in three liters of solution and aerated to insure optimum conditions for metabolism (Hoagland and Broyer²).

(b) *Decapitated Plants.* The shoots were cut off $\frac{3}{4}$ inches above the seed hull. Only the roots were dipped into the solution which was contained in shallow Pyrex trays.

(c) *Entire Plants.* The plants were left undisturbed, and roots and shoot were analyzed separately.

In all experiments listed under (a), (b) and (c) the roots were left in the solutions for a period of 10 hours. The loss of K was ascertained by analysis of the plant material using the cobaltinitrite method.³

(d) *Experiments with Radioactive Potassium.* The root systems of entire plants were kept for a few hours in KNO_3 solutions containing radioactive K. As soon as the shoot had accumulated substantial amounts of the radioactive element, the plants were transferred to the desired solutions. The outgo of K was determined by radioactive analysis of the solutions (Geiger counter).

Behavior of Roots in Distilled Water.—At the outset it was necessary to determine the loss of K when roots were placed in distilled water. For excised roots the data given in the table below are representative. The K content of the root is expressed as milliequivalents of K per 100 g. of dry roots. In all cases the losses are small and within experimental error. Special attention should be called to the last two rows of the table giving data for roots continuously leached for 10 hours with distilled water at a rate of ten gallons per hour. Nevertheless, the roots maintained their potassium level.

DRY WEIGHT OF ROOTS (168 PLANTS)	K CONTENT OF ROOTS M. E./100 G.	DIFFERENCE, PER CENT	REMARKS
3.66	43.1	...	Untreated
3.76	42.5	-1.4	In 3 liters H_2O
3.81	42.3	-1.9	In 3 liters H_2O
3.99	42.2	-2.1	In 100 gallons H_2O
3.55	41.4	-4.0	In 100 gallons H_2O

Similar tendencies were observed for plants which were allowed to accumulate radioactive K and then were kept in distilled water (7 plants in 400 cc.) for 4-7 hours. The radioactivity of 25 cc. of water corrected for background and radioactive decay and expressed in terms of "counts per minute" recorded by the Geiger counter is as follows:

$$\text{H}_2\text{O (3.72 hour period)} = 8.1 \pm 3.82$$

$$\text{H}_2\text{O (6.53 hour period)} = 6.6 \pm 5.36$$

The loss of radioactive K is small, less than 0.17 per cent and is statistically not significant. Evidently the low salt roots under investigation have a pronounced capacity to retain potassium against distilled water.

Behavior of Roots in Salt Solutions.—Since clays carry adsorbed cations, particularly Na, NH_4 , Ca or Mg, the question naturally arises whether these ions *per se* exert some specific influence on the condition of the root in relation to its power of retention of potassium. Accordingly, excised roots were kept for 10 hours in three liters of the following solutions containing 3-5 milliequivalents per liter: NaCl, NaHCO_3 , NH_4HCO_3 , CaCl_2 and MgCl_2 . In all cases the loss of K was small and did not exceed 4 per cent, which is still within experimental error. A HCl solution which was maintained at pH = 4.1 lowered the K content of the root by 5.1 per cent, a value which probably is significant.

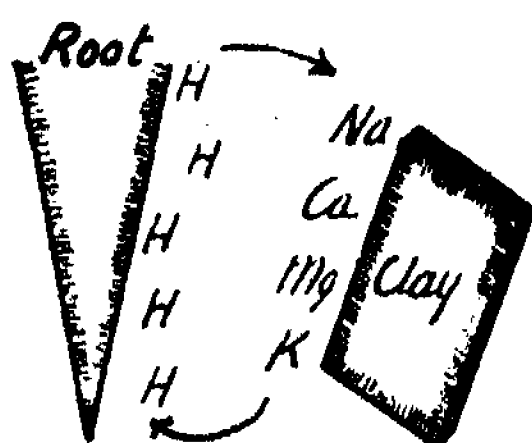
A different picture is revealed by the more sensitive radioactive technique applied to entire plants. Their roots were kept for 11 hours in 900 cc. of various electrolyte solutions containing 5 milliequivalents of cations per liter and, in each case, 0.5 milliequivalents CaCl_2 . The following counts above background were obtained on 25 cc. of solution (background = 19.8 ± 1.62):

H_2O	=	8.9 \pm 7.00	KCl	=	42.8 \pm 9.78
LiCl	=	-6.9 \pm 7.22 ⁴	KNO_3	=	47.2 \pm 9.50
NaCl	=	-11.5 \pm 7.34	K_2SO_4	=	40.4 \pm 9.78
KCl	=	62.5 \pm 10.5	KH_2PO_4	=	47.1 \pm 10.80
NH_4Cl	=	188.8 \pm 17.7	KHCO_3	=	35.7 \pm 10.67
HCl	=	219.4 \pm 11.75			

A lyotropic series of monovalent cations for the removal of radioactive K from roots is indicated. The values for H_2O , LiCl and NaCl fall within the variability of the background (cosmic rays) and are not significant. In the cases of NH_4Cl and HCl the relatively high K outgo may have been in part a consequence of injurious effects of high electrolyte concentrations. A corresponding set with K salts (3.0 milliequivalents per liter) showed that inorganic anions which are common to nutrient solutions exerted no specific influence. The counts from systems containing MgCl_2 , CaCl_2 , SrCl_2 or BaCl_2 did not exceed the background. Regarding the actual

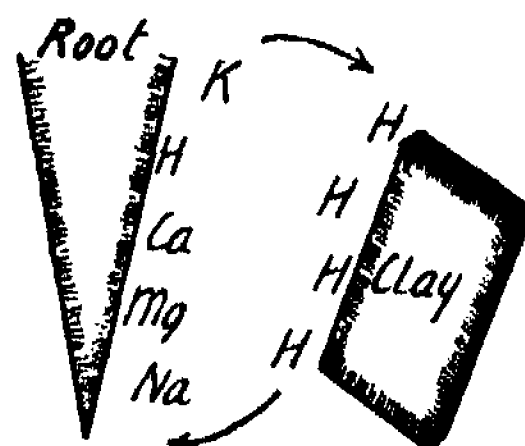
amounts of K involved, no definite figure can be given, except that the minimum amount of K lost by the roots is 0.000213 milliequivalents per 25 cc. per 100 counts.

Behavior of Roots in Colloidal Clay Suspensions.—The suspensions used in this study were of bentonite clay and of Yolo clay, both consisting of the common clay mineral montmorillonite. The bentonites were electro-dialyzed and then converted into the desired basic clays by addition of hydroxides, according to the equation $\text{H-clay} + \text{NaOH} = \text{Na-clay} + \text{HOH}$. The Yolo clays were subjected to leaching with neutral salts. The cation adsorption capacity (base exchange capacity) was 100 milliequivalents per 100 g. for the bentonites and 55 milliequivalents per 100 g. for the Yolo colloids as determined by the ammonium acetate method.



Contact intake

FIGURE 3



Contact depletion

FIGURE 4

If excised roots are immersed in H or Ca-H bentonite suspensions for ten hours they suffer heavy losses of K as seen from the following data:

SYSTEM	CLAY CONCENTRATION, PER CENT	INITIAL pH OF SUSPENSION	K IN ROOTS M. E./100 G.	DIFFERENCE DUE TO COLLOID, PER CENT
H ₂ O	45.9
H-bentonite sol	3.97	3.05	4.7	-89.8
H-bentonite flakes	5.17	3.70	34.3	-26.0
K-H-bentonite sol	0.83	4.90	59.0	+28.5
Ca-H-bentonite sol	0.48	5.65	37.0	-19.4

The roots which lost 90 per cent of their total potassium showed indications of injury. From K-H-bentonite the roots accumulate K but lose Ca to the extent of 22.2 per cent. From Ca-H-clay they take up Ca ions (6.4 per cent) but lose K. The intake and outgo of nutrient ions is, therefore, of a differential nature. Coarse suspensions (flakes) are less effective than highly dispersed sols, as would be expected if contact phenomena are involved.

Furthermore, if excised roots are separated from H-bentonite suspensions (pH = 3.58) by a membrane which permits easy passage to ions but bars colloidal particles from contact with roots, no losses of K occur.

Likewise, if excised roots are suspended in a positive iron hydroxide sol (0.33 per cent) the lowering of the K level of the roots amounts to only 4.4 per cent. This is in harmony with the contact exchange theory according to which the negative barley roots⁶ should not transfer cations to positive colloids.

Experiments with plants which were decapitated $\frac{3}{4}$ inches above the base of the shoot fully corroborate the results obtained with excised roots, as shown in the following table:

SYSTEM	CLAY CONCENTRATION, PER CENT	INITIAL pH OF SUSPENSION	REDUCTION OF K CONTENT OF ROOTS, PER CENT
H-bentonite	0.53	3.85	26.8
Na-bentonite	0.48	7.35	14.8
NH ₄ -bentonite	0.41	7.25	32.6
Ca-bentonite	0.31	7.40	0.9

It appears that the nature of the adsorbed cation, rather than the pH of the suspension regulates the magnitudes of the K extraction by the colloidal particles; Ca-clay, in the concentrations used, did not influence the K status of the root systems.

Turning to radioactive experiments, the clay sols were compared with salt solutions, both systems containing equal amounts of cations. For 7 plants in 900 cc. liquid (5.0 milliequivalents cation + 0.5 milliequivalents CaCl₂ per liter) the following counts above background were obtained from 25 cc. solution after a contact period of 53 hours:

H ₂ O = 7.1 ± 6.45	
NaCl = 4.2 ± 6.41	NH ₄ Cl = 668 ± 49.8
Na-Yolo colloid = 606 ± 92.5	NH ₄ -Yolo colloid = 1044 ± 62.2

Again the colloidal solutions are more effective than the true solutions, particularly in the case of the Na systems. With an improved technique of counting, the following measurements were made on divalent systems (2.2 milliequivalents cations per liter) which had 7 plants in 400 cc. solution for a period of 3.7 hours (counts above background, on 25 cc. solution):

H ₂ O = 8.1 ± 3.82	CaCl ₂ = 11.3 ± 3.68
MgCl ₂ = 3.3 ± 4.06	CaCO ₃ = 17.0 ± 3.50
Mg-Yolo colloid = 109 ± 6.44	Ca-Yolo colloid = 66.3 ± 3.81

The greater efficiency of the clay systems as compared with the chloride salt solutions for extracting radioactive K from the roots is beyond doubt.

The influence of salt concentration and clay concentration on the removal of radioactive K from roots is illustrated in figure 5. The comparisons include KCl, CaCl₂, K-Yolo and Ca-Yolo colloids. Two features are outstanding. First, the effects of monovalent cations exceed those of the divalent cations; second, the clay systems uniformly give higher counts

than the salt solutions. These data convincingly prove the importance of root-colloid effects. The results obtained with the radioactive technique fully substantiate the observations made with excised roots and decapitated plants.

Injury Tests.—The root systems of entire plants were immersed in H-bentonite sols (0.16 per cent) until they had lost 20.0 per cent of their total potassium. When these roots were transferred to nutrient solutions and

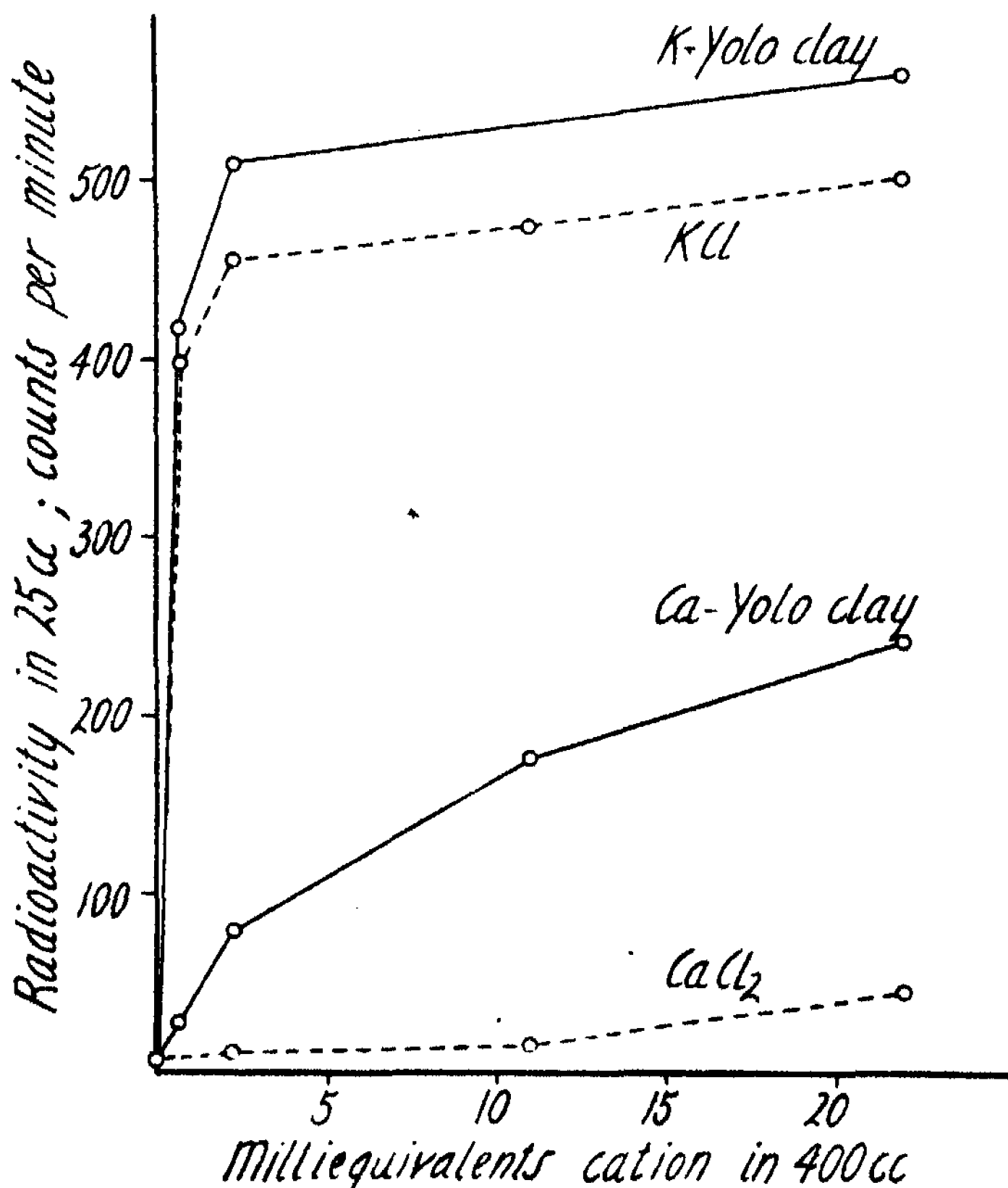


FIGURE 5

kept there for 12 hours they accumulated K to the extent of 153 per cent of their original K content. Apparently the clay treatment had not impaired the capacity for salt accumulation; in fact, the roots which had been in the bentonite sol proved to be just as efficient absorbers as the control roots which were not in contact with the clay.

A set of radioactive K roots (84 plants) were kept in a K-Ca-clay suspension for six hours, and during this time they continuously lost large

amounts of radioactive K. When these plants were transferred to distilled water the outgo of radioactive K immediately fell to such small magnitudes that the counts of the water did not exceed those of the background. It must be concluded that if any root injury occurs it can hardly be attributed to contact as such; rather it must be considered a consequence of resulting disturbances of the electrolyte balance within the plant system.

New Aspects of Plant Nutrition.—The barley plants grown by the technique referred to in this article possess an inherent capacity for pronounced accumulation of various nutrient cations. The radioactive tests reveal that during intake there occurs, simultaneously, a release of nutrient cations to the surrounding medium.

Particularly striking are the observations with radioactive K roots immersed in KCl or K-clay (figure 5). Potassium from the nutrient medium moves into the plant whereas K contained in the root is migrating out. In the case of 10 per cent K-clay (figure 5) the roots transferred about 10 per cent of their radioactivity to the clay particles.

Quantitative insight is gained from the following crucial experiment. Eighty-four radioactive plants were placed for 5 hours in a K-Ca-bentonite suspension (0.35 per cent). The roots weighed 4.51 g. (oven dry) and contained initially 1.685 milliequivalents K. At the end of the experiment they contained 2.840 milliequivalents K, a gain of 1.155 milliequivalents or 68.3 per cent. This latter value represents only the net intake. Actually the roots must have absorbed larger amounts of K because they simultaneously yielded at least 0.114 milliequivalents K to the clay, as indicated by the outgo of radioactive potassium.

In view of these findings, our concepts of the mechanism of mineral absorption by plants must be modified and extended. The results show that the intake of ions is not an uni-directional process; ions of the same species may move into the root and out of the root at the same time. The outgo is especially pronounced when the roots are in contact with colloidal systems. Accumulation and depletion are only net effects of ionic movements.

Acknowledgments.—The authors are indebted to Professor D. R. Hoagland for his interest and valuable suggestions, to Professor E. O. Lawrence, Director of the Radiation Laboratory, for supplying radioactive potassium and to Mr. A. D. Ayers for technical assistance.

A detailed account of the experiments will be published in *Soil Science*.

¹ Jenny, H., *Jour. Phys. Chem.*, **40**, 501 (1936).

² Hoagland, D. R., and Broyer, T. C., *Plant Physiol.*, **11**, 471 (1936).

³ Hibbard, P. L., and Stout, P. R., *Jour. Assoc. Offic. Agric. Chem.*, **26**, No. 1, 137 (1933).

⁴ This negative value was obtained as follows: Ten readings of the background gave

a value of 19.8 ± 1.62 . Ten readings of 25 cc. of LiCl solution gave a count of 17.7 ± 1.62 which is within the variability of the background. The difference, -2.2 ± 2.29 multiplied by the radioactive decay factor of 3.15 gives the tabulated value, -6.9 ± 7.22 .

* That the roots are negative is indicated by measurements of streaming potentials, and moreover by the fact that positive particles of iron hydroxide strongly adhere to the root while negative clay particles do not show such a behavior. The dried, ground roots have a cation adsorption capacity of about 11 milliequivalents per 100 g. as determined by the ammonium acetate method.

ON LOCALLY CONNECTED SETS AND RETRACTS

BY S. LEFSCHETZ

DEPARTMENT OF MATHEMATICS, PRINCETON UNIVERSITY

Communicated July 23, 1938

In the present Note we propose to summarize a few results on locally connected sets and retracts. For the definitions we refer the reader to an earlier paper.¹ By way of notation we designate here by LC^* , LC^{**} the types designated by LC , \overline{LC} , loc. cit. Likewise instead of *semi-singular* complex, we shall say *partial realization* of a complex. The comparison with the retracts (loc. cit., Theorem II) reads now: $LC^* = ANR$, $LC^{**} = AR$.

A closed locally finite (with finite stars) simplicial geometric complex \mathfrak{R} with countable elements $\sigma^1, \sigma^2, \dots$ is said to be *regular* whenever $\text{diam. } \sigma^i \rightarrow 0$ with $1/i$. Similarly a partial or full realization L of \mathfrak{R} on a metric space \mathfrak{R} is *regular* whenever if ζ^i is the sum of the faces of σ^i already realized, $\text{diam. } \zeta^i \rightarrow 0$ with $1/i$. A continuous complex K which is the extension of L to \mathfrak{R} minus a finite closed sub-complex is said to be an *almost full realization* of \mathfrak{R} .

In the sequel \mathfrak{R} shall designate a compact metric space.

THEOREM 1. *N. a. s. c. for \mathfrak{R} to be LC^p is that every regular partial realization of a complex such as \mathfrak{R} , where $\dim \mathfrak{R} \leq p$, may almost be extended to a full realization K of \mathfrak{R} . For LC^* the result is the same without dimensional restriction, and likewise for LC^{**} except that K must be a full realization.*

If we compare with Theorem I of our paper and the LC^* , LC^{**} definitions, we find that the ϵ , η conditions are replaced by the "structure" of the infinite complexes.

Let now \mathfrak{R} be a finite simplicial geometric complex and let U map \mathfrak{R} on \mathfrak{R} , thus giving rise to a continuous complex K on the latter. We assign to K the dimension of \mathfrak{R} . Let now A be a set on \mathfrak{R} and let it be mapped by W on the set A' of the same space. If there exists a mapping $V: A \rightarrow \mathfrak{R}$

such that $W = UV$, A is said to be *transformed* onto K on \mathfrak{R} . If W is a deformation A is said to be *deformed* onto K over \mathfrak{R} (ϵ deformed when W is an ϵ deformation). These definitions are readily shown to depend solely upon K and not upon the particular antecedent \mathfrak{R} of K chosen.

THEOREM 2. *When \mathfrak{R} is LC^p , every closed subset of \mathfrak{R} whose dimension $q \leq p$, may be ϵ deformed whatever ϵ into a continuous complex whose dimension $\leq q$.*

THEOREM 3. *N. a. s. c. for \mathfrak{R} to be LC^* is that the space be ϵ deformable whatever ϵ into a finite continuous complex. The N. a. s. c. for LC^{**} are the condition just stated plus contractibility.*

The last two theorems bear considerable analogy with the "polytope" theorems of Alexandroff. We may also extend Theorem III of our paper, and many similar results, by the substitution of an LC^{**} space for the Hilbert parallelotope. Finally the same theorems, suitably reworded if need be, in terms of chain-deformation, may be extended to the HLC class.²

¹ S. Lefschetz, *Ann. Math.*, **35**, 118-129 (1934). The LC^* spaces are those for which the extension of Theorem I of the paper may be carried out with a fixed $\eta(\epsilon)$, independent of p . The LC^{**} spaces are those in which in addition the extension may always be carried out without regard to the meshes of the complexes. That is to say any partial realization may be extended to a full realization.

² For the relation between the LC and HLC classes, see S. Lefschetz, *Duke Jour.*, **1**, 1-19 (1935); **2**, 435-442 (1936).

CONFORMAL GEOMETRY OF HORN ANGLES OF SECOND ORDER

BY EDWARD KASNER AND JOHN DE CICCIO

DEPARTMENTS OF MATHEMATICS, COLUMBIA UNIVERSITY AND BROOKLYN COLLEGE

Communicated August 12, 1938

I. Introduction.—In preceding papers,¹ a study of the conformal geometry in a plane of horn angles of first order was made. In this paper, we wish to develop the conformal geometry of horn angles of second order. In the earlier papers, a fundamental differential conformal invariant of the third order was found for a horn angle of first order. In our new work, we shall study a fundamental conformal invariant of the fifth order for a horn angle of second order.

The total geometry of horn angles is non-archimedian: for each order of horn angle we have to introduce a new special Finsler metric, the appropriate Finsler space increasing in dimensionality and having a more complicated distance element as the order increases.

A *horn angle of second order* consists of an ordered pair of curves which pass through a given point in a common direction and which possess the same curvature but distinct rates of variation of curvature at the given point. Thus the curves of a horn angle of second order have three (but *not* four) consecutive points in common at the given point. In our previous work,² it was shown that a *horn angle of second order possesses a unique absolute conformal invariant M_{12} of the fifth order (which depends only on the first five derivatives of the two curved sides of the horn angle at the given point)*. By this, it is meant that two given horn angles of the second order can be transformed into one another by a *formal* conformal transformation if and only if the two horn angles have the same M_{12} (and the sign of $\gamma_2' - \gamma_1'$ is the same for both horn angles). From this theorem, we see that, in order to study the conformal geometry of horn angles of second order, it is necessary merely to study the conformal geometry of differentiable elements of the fifth order which pass through a given point in a given direction and which have the same curvature.

A *differential element of the fifth order* is, of course, defined by a point and the first five successive derivatives at that point, that is, by a set of seven numbers. In the remainder of this paper, whenever we speak of a *curve*, we shall mean a differentiable element of the fifth order. By a *horn set* (γ), we shall mean the totality of all curves (fifth order elements) which pass through a given point in a common direction and which possess the same curvature γ . Let x be the first derivative of the curvature γ with

respect to the arc length (that is, $x = \frac{d\gamma}{ds}$) of any curve C of the horn-set

(γ), y the second derivative of the curvature γ (that is, $y = \frac{dx}{ds} = \frac{d^2\gamma}{ds^2}$)

and z the third derivative of the curvature γ (that is, $z = \frac{dy}{ds} = \frac{d^2x}{ds^2} = \frac{d^3\gamma}{ds^3}$).

Then any curve C of a horn-set (γ) may be defined by the three numbers (x, y, z) . Thus we can think of a horn-set (γ) as being a three dimensional space K_3 where any point of K_3 is a curve C of the horn-set (γ).

We shall call any arbitrary set of ∞^1 curves of a horn-set (γ) a *series of the horn-set* (γ). Thus any series of a horn-set (γ) is given by the two equations $y = y(x)$, $z = z(x)$, where y and z are arbitrary functions of x . A special type of series is the *linear series*. By a *linear series*, we mean any series whose equations are $y = px + r$, $z = qx + s$, where p, q, r, s are constants. Thus a linear series consists of ∞^1 curves of a horn-set (γ)

whose first derivative $x = \frac{d\gamma}{ds}$, second derivative $y = \frac{d^2\gamma}{ds^2}$ and third deriva-

tive $z = \frac{d^3\gamma}{ds^3}$ of the curvature γ with respect to the arc length satisfy two linear equations.

We term an arbitrary set of ∞^2 curves of a horn-set (γ) a *congruence of the horn-set* (γ) . Thus any congruence of the horn-set (γ) is given by the single equation $z = z(x, y)$, where z is an arbitrary function of x and y . A special kind of congruence is the flat congruence. By a *flat congruence*, we mean any congruence whose equation is $z = ax + by + c$, where a, b, c are constants. Thus a flat congruence consists of ∞^2 curves of a horn-set (γ) whose first derivative $x = \frac{d\gamma}{ds}$, second derivative $y = \frac{d^2\gamma}{ds^2}$ and third derivative $z = \frac{d^3\gamma}{ds^3}$ of the curvature γ with respect to the arc length satisfy a single linear equation.

It is found that the group of conformal transformations induces a *fundamental five-parameter group* G_5 between the curves of a horn-set (γ) and the curves of a second horn-set (Γ) . It is our purpose to study the geometry of this fundamental group G_5 . Under G_5 , linear series are transformed into linear series and flat congruences are converted into flat congruences. It is found that a *horn-set* (γ) is an *affine three-space* K_3 . In this paper, we shall obtain the elementary conformal invariants of the curves, linear series and flat congruences of a horn-set (γ) .

II. *The Fundamental Group* G_5 .—Let C be any curve and let D be the transformed curve under any conformal transformation. Upon finding the first derivative $X = \frac{d\Gamma}{dS}$, the second derivative $Y = \frac{d^2\Gamma}{dS^2}$ and the third derivative $Z = \frac{d^3\Gamma}{dS^3}$ of the curvature Γ with respect to the arc length S of the transformed curve D in terms of the first derivative $x = \frac{d\gamma}{ds}$, the second derivative $y = \frac{d^2\gamma}{ds^2}$ and the third derivative $z = \frac{d^3\gamma}{ds^3}$ of the curvature γ with respect to the arc length s of the curve C , it is found after lengthy calculations (using either the method of power series, or of successive differentiation of the Cauchy-Riemann equations) that any curve $C(x, y, z)$ of the horn-set (γ) is transformed into a curve $D(X, Y, Z)$ of the horn-set (Γ) , which is given by the equations

$$\begin{aligned} X &= m^2x + h, \\ Y &= m^3y + 2mnx + k, \\ Z &= m^4z + 5m^2ny + (5n^2 + \Gamma^2m^2 - \gamma^2m^4)x + l, \end{aligned} \tag{1}$$

where $m \neq 0$, h, k, l are constants. Thus the group of conformal transformations induces a *five-parameter group* G_5 between the curves of the horn-

set (γ) and the curves of the horn-set (Γ) . This is our fundamental group G_b . From the equations (1), it is immediately obvious that a horn-set (γ) is an affine three-space.

III. *The Conformal Measure M_{12} of a Horn Angle of Second Order.*—

THEOREM 1. Under G_b , any curve of a horn-set (γ) can be transformed into any other curve of a horn-set (Γ) . A horn angle of second order (that is, an ordered pair of curves of a horn-set (γ)) has the unique absolute differential conformal invariant³

$$M_{12} = \frac{(x_2 - x_1)^3}{4(x_2 - x_1)(z_2 - z_1) - 5(y_2 - y_1)^2 - 4\gamma^2(x_2 - x_1)^2}; \quad (2)$$

also the sign of $x_2 - x_1$ is invariant.

Theorem 1 is an immediate consequence of the equations (1). A complete proof of this result is given in the paper by Kasner, "The Two Conformal Invariants of Fifth Order" (loc. cit). By means of Theorem 1, we define M_{12} as the *conformal measure* of the horn angle of second order. Thus any horn-set (γ) is a special type of Finsler three-space with the special Finsler metric⁴

$$ds = \frac{dx^3}{4xdz - 5dy^2 - 4\gamma^2 dx^2}. \quad (3)$$

This metric is non-riemannian and therefore non-euclidean. We define the length of arc of a series $y = y(x)$, $z = z(x)$ of a horn-set (γ) as the integral

$$s = \int \frac{dx}{4z' - 5y'^2 - 4\gamma^2}. \quad (4)$$

IV. *The Null Series of a Horn-Set (γ) .*—We shall call a series of a horn-set (γ) a *null series* if the conformal measure of any horn angle of the series is either zero or infinity or indeterminate. From (2) or (3), it follows immediately that the null series of a horn-set (γ) are given by the Monge differential equation

$$4z' - 5y'^2 - 4\gamma^2 = 0; \quad (5)$$

together with the exceptional linear series

$$x = \text{constant}. \quad (6)$$

V. *The General Linear Series.*—A linear series is said to be a *general linear series* if it is not tangent to a null series (that is, the general linear series and the null series cannot have two consecutive curves in common). Thus, the linear series

$$y = px + r, \quad z = qx + s, \quad (7)$$

is a general linear series if and only if

$$4q - 5p^2 - 4\gamma^2 \neq 0. \quad (8)$$

From (1), we find that *under G_h , any general linear series of a horn-set (γ) is converted into a general linear series of a horn-set (Γ)* . The transformation between the linear series of the two horn-sets is given by the equations

$$\begin{aligned} P &= mp + \frac{2n}{m}, \\ Q &= m^2q + 5np + \frac{5n^2}{m^2} + \Gamma^2 - m^2\gamma^2, \\ R &= m^3r - hmp - \frac{2nh}{m} + k, \\ S &= m^4s + 5m^2nr - h(m^2q + 5np + \frac{5n}{m^2} + \Gamma^2 - \gamma^2m^2) + l. \end{aligned} \quad (9)$$

From (9), we find

THEOREM 2. *Two general linear series of two horn-sets are equivalent to one another under G_h if and only if the sign of*

$$4q - 5p^2 - 4\gamma^2 \neq 0, \quad (10)$$

is the same for both linear series. Two general linear series of a given horn-set have three independent invariants

$$\frac{(p_2 - p_1)^2}{4q_2 - 5p_2^2 - 4\gamma^2}, \quad (11)$$

$$\frac{(p_2 - p_1)^2}{4q_1 - 5p_1^2 - 4\gamma^2} \quad (12)$$

$$\frac{(p_2 - p_1)(s_2 - s_1) - (q_2 - q_1)(r_2 - r_1)}{(4q_1 - 5p_1^2 - 4\gamma^2)^{5/2}}. \quad (13)$$

For two intersecting general linear series (that is, the two series have a curve C in common) possess the two independent invariants (11) and (12).

In the special case where $p_2 - p_1 = 0$, we find that the ratio (12)/(11) is the appropriate invariant. A similar remark applies to Theorems 4 and 6.

According to Theorem 2, we now define the *direct dihorn angle* α_{ij} between the ordered pair of general linear series (L_i, L_j) by the formula

$$\alpha_{ij} = \frac{(p_j - p_i)^2}{4q_j - 5p_j^2 - 4\gamma^2} \quad (14)$$

The reverse dihorn angle α_{ji} for the ordered pair of general linear series (L_i, L_j) is defined by the formula (14) where i and j are interchanged. It is found that α_{ij} and α_{ji} are entirely independent quantities.

From equations (1) and (9), we obtain

THEOREM 3. *A point and a general linear series possess the two independent invariants*

$$\frac{y - px - r}{(4q - 5p^2 - 4\gamma^2)^{3/2}}, \quad (15)$$

$$\frac{2(z - qx - s) - 5p(y - px - r)}{(4q - 5p^2 - 4\gamma^2)^2}. \quad (16)$$

We omit the full proofs which are rather long.

VI. *The General Flat Congruence.*—A flat congruence is termed a *general flat congruence* if it is not the osculating flat congruence of any null series (that is, the flat congruence and the null series cannot have three consecutive curves in common). Thus the flat congruence

$$z = ax + by + c, \quad (17)$$

is a general flat congruence if and only if

$$5a + b^2 - 5\gamma^2 \neq 0. \quad (18)$$

By (1), we find that *under G_h , any general flat congruence of a horn-set (γ) is converted into a general flat congruence of a horn-set (Γ)* . This correspondence between the general flat congruences of two horn-sets is given by the equations

$$\begin{aligned} A &= m^2a - 2nb - \frac{5n^2}{m^2} + \Gamma^2 - m^2\gamma^2, \\ B &= mb + \frac{5n}{m}, \end{aligned} \quad (19)$$

$$C = m^4c + (2hn - mk)b - hm^2a + \frac{5hn^2}{m^2} - \frac{5kn}{m} - h\Gamma^2 + hm^2\gamma^2 + l.$$

By equations (19), we find

THEOREM 4. *Two general flat congruences of two horn-sets are equivalent to one another if and only if the sign of*

$$5a + b^2 - 5\gamma^2 \neq 0, \quad (20)$$

is the same for both flat congruences. The general flat congruences possess the two independent invariants

$$\frac{(b_2 - b_1)^2}{5a_2 + b_2^2 - 5\gamma^2}, \quad (21)$$

$$\frac{(b_2 - b_1)^2}{5a_1 + b_1^2 - 5\gamma^2}. \quad (22)$$

We define the *direct dihorn angle* β_{ij} between the ordered pair of flat congruences (P_i, P_j) by the formula

$$\beta_{ij} = \frac{(b_j - b_i)^2}{5a_j + b_j^2 - 5\gamma^2}. \quad (23)$$

The *reverse dihorn angle* β_{ji} is defined by the formula (23) where we interchange i and j . It is obvious that β_{ij} and β_{ji} are entirely independent quantities.

From equations (1) and (19), we find

THEOREM 5. *A curve and a general flat congruence possess the unique invariant*

$$\frac{25(z - ax - by - c)}{(5a + b^2 - 5\gamma^2)^2}. \quad (24)$$

The formula (24) gives the extremum (minimum or maximum) conformal measure between the given curve and the curves of the general flat congruence.

From equations (9) and (19), we obtain

THEOREM 6. *A general linear series and a general flat congruence possess the two unique invariants*

$$\frac{(5p - 2b)^2}{5a + b^2 - 5\gamma^2}, \quad (25)$$

$$\frac{(5p - 2b)^2}{4q - 5p^2 - 4\gamma^2}. \quad (26)$$

VII. In a later paper, we shall study the *Trihornometry of Second Order*. This is a study of the relationships between the conformal invariants of a trihorn of second order. By a *trihorn of second order*, we mean an ordered triplet of curves (C_1, C_2, C_3) which pass through a given point in a given direction such that every two curves (C_i, C_j) have three consecutive points (but not four) in common at the given point. It is found that a trihorn of second order contact possesses nine invariants: the three conformal measures M_{12}, M_{23}, M_{31} , the three direct dihorn angles $\alpha_{12}, \alpha_{23}, \alpha_{31}$ and the three reverse dihorn angles $\alpha_{21}, \alpha_{32}, \alpha_{13}$. These nine quantities are interdependent. We find laws analogous to the identity relation for the angles of a triangle in euclidean geometry, the law of sines and the law of cosines. In general

it is found that four parts of a trihorn have to be given in order to determine the remaining five parts. This is entirely different from ordinary euclidean geometry and trihornometry of first order contact,³ where only three parts are necessary to determine the remaining three parts.

¹ Kasner and Comenetz, "Conformal Geometry of Horn Angles," *Proc. Nat. Acad. Sci.*, 22, No. 5, 303-309 (1936); Kasner, "Fundamental Theorems of Trihornometry," *Sci.*, 85, No. 2211, 480-482 (1937); Kasner, "Trihornometry: A New Chapter of Conformal Geometry," *Proc. Nat. Acad. Sci.*, 23, No. 6, 337-341 (1937).

² Kasner, "Conformal Geometry," *Proc. Fifth Internat. Congr. Math.*, Cambridge, 2, 81 (1912); Kasner, "The Two Conformal Invariants of Fifth Order," *Trans. Amer. Math. Soc.* (1938) and "Schwarzian Symmetry," *Annals of Math.*, 1938.

³ This is contrasted with a horn angle of first order which possesses the unique absolute conformal invariant of the third order $\frac{(x_2 - x_1)^2}{y_2 - y_1}$ where x denotes the curvature and y denotes the rate of variation of curvature at the given point.

⁴ For horn angles of first order, we obtain an associated Finsler plane with the special Finsler metric $ds = \frac{dx^2}{dy}$. See Comenetz, "Kasner's Invariant and Trihornometry," *Amer. Math. Monthly*, 45, 81-87 (1938). This metric is probably the *simplest conceivable example of a Finsler space* which is not merely riemannian.

⁵ Kasner, "Trihornometry, a New Chapter in Conformal Geometry," loc. cit.

A SKELETON LIFE TABLE

BY CARL R. DOERING AND ALICE L. FORBES

HARVARD SCHOOL OF PUBLIC HEALTH, DEPARTMENT OF VITAL STATISTICS

Communicated August 11, 1938

Life tables are being used considerably in vital statistics, notably by Raymond Pearl, Louis Dublin and Alfred Lotka. The accepted method of computation of life tables is the careful actuarial method used by the United States Bureau of the Census, and the Metropolitan Life Insurance Company, but many short methods of computation have been proposed with the idea of getting more quickly and easily a good idea of the expectations and of other life table functions at various ages: such are Yule's short method involving exponentials¹ and King's abridged method.² However, these so-called short methods still involve long computations, and often call for preliminary smoothing of the original data, which are usually too time-consuming to permit a health officer to apply the technique to local data. The method presented here seems to give very good results with remarkably few age groups, and in addition is very simple to compute.

We start with the age specific death rates, and all the rest of the work is derived from these. Let m_x^{x+h} be the per capita death rate for the age group x to $x+h$ years. We choose arbitrarily a value l_0 , the number alive at age zero in a cohort. Then we compute L_0^{0+h} , the stationary population in that group; d_0^{0+h} , the deaths in the cohort during these years; and l_0+h the survivors at age $0+h$; and repeat the process throughout the table. In all intervals except the first and last we take

$$L_x^{x+h} = \frac{l_x}{\frac{1}{h} + \frac{1}{2} m_x^{x+h}}; \quad d_x^{x+h} = m_x^{x+h} L_x^{x+h}; \quad l_{x+h} = l_x - d_x^{x+h}.$$

The $\frac{1}{2}$ comes from an assumption that the deaths in an interval are spread evenly throughout the interval. Between the ages of 5 and 75 this is good enough, but in other cases it is not, and when it is not, we use

$$L_x^{x+h} = \frac{l_x}{\frac{1}{h} + (1-c)m_x^{x+h}}$$

where c is the fraction of the interval lived by those who die within the interval. This is most often used in the age group "under 5."

If the first age group is "under 1," the same formula can be used, but populations under one are apt to be poorly reported, giving too high death rates, so a simple alternative is employed. The births are used instead of the enumerated population, which divided into the deaths under one give the infant mortality. Consequently

$$d_0^1 = l_0 \times \text{I. M. (per capita)}, \text{ and } l_1 = l_0 - d_0^1$$

so we can proceed. But we shall need L_0^1 and that is equal to $l_1 + cd_0^1$.

In the last age group, it is obvious that $d_x^\infty = l_x$, so it is clear that

$$L_x^\infty = \frac{l_x}{m_x^\infty}$$

if we assume that the population from age x on can be regarded as stationary. This enables one to fill in the table. The necessary values of c are obtained from a study of the distribution of deaths by months for under one, or by single years for under five, but once determined for a given year and general area, need not be recomputed. For example, if we want to compute a life table for a county of New York State in 1930, we can get a good estimate of c from the average age of deaths of infants or small children for the total New York State, and then apply it to our county, and it will be good enough.

With the columns l_x , L_x^{x+h} , d_x^{x+h} filled in, we form a column T_x , for the

populations above age x by summing all L_x^{x+h} from the bottom up, and then the expectation $\dot{e}_x = T_x/l_x$.

A numerical example may make this clearer (see table 1).

TABLE 1

NEW YORK STATE, ONONDAGA COUNTY, 1929-31, DEATHS IN INSTITUTIONS REMOVED

AGE	CENSUS POPUL. 1930	AVERAGE DEATHS 1929-31	m_x^{x+h}	l_x	L_x^{x+h}	d_x^{x+h}	T_x	\dot{e}_x
0-1	4,950*	279.67	0.0565†	1000	953‡	56	61,322	61.32
1-4	18,835	75.00	0.00398	944	3,744	15	60,369	63.98
5-9	26,138	42.33	0.00162	929	4,624	7	56,625	60.98
10-14	25,761	34.00	0.00132	921	4,590	6	52,000	56.45
15-19	23,557	47.67	0.00202	915	4,552	9	47,410	51.81
20-24	23,842	56.00	0.00235	906	4,503	11	42,858	47.31
25-29	22,712	64.00	0.00282	895	4,445	13	38,355	42.84
30-34	23,203	72.33	0.00312	883	4,379	14	33,910	38.42
35-44	45,593	259.00	0.00568	869	8,451	48	29,531	33.98
45-54	34,559	407.67	0.0118	821	7,753	91	21,080	25.67
55-64	23,823	594.33	0.0249	730	6,487	162	13,327	18.27
65-74	13,538	721.00	0.0533	568	4,484	239	6,840	12.05
75+	5,348	746.67	0.140	329	2,356	329	2,356	7.16
	291,859	3399.67			61,322	1000		

* Average births for 1929-31. † Infant mortality. ‡ $c = .18$

NOTE: These figures are cut down from the original computation in which more places were carried.

We started with a radix of 1000. Then $1000 \times .0565 = 56$ deaths. $1000 - 56 = 944$. To get L_0^1 , we took $944 + .18 \times 56 = 953$. From here on we proceeded by the regular formula, with $L_1^4 = \frac{944}{.25 + .00199} = 3744$; $d_1^4 = 3744 \times .00398 = 15$; and $l_5 = 944 - 15 = 929$; and so on. When we got $l_{75} = 329$, we immediately wrote $d_{75}^\infty = 329$, and then $L_{75}^\infty = \frac{329}{.140} = 2356$. $T_{75} = 2356$. $T_{65} = 2356 + 4484 = 6840$. $T_{55} = 6840 + 6487 = 13,327$, and so on, adding from the bottom up. $\dot{e}_0 = \frac{61,322}{1000} = 61.32$. $\dot{e}_1 = \frac{60,369}{944} = 63.98$. The sum of the d_x^{x+h} column must equal l_0 , and the sum of the L_x^{x+h} must equal T_0 .

The question now arises as to how good the results are—how close do they come to the results of the detailed actuarial methods? The published life tables of the United States Census for 1910 and 1920 give the raw data from which they were computed, so we can compare our work with these tables, and also with other short methods. Because King's method is

constructed for five year intervals, and Yule feels that his is valid only for five year intervals, we shall use these small age groups first, combining into larger groups later. The published figures for 1910 are computed by single years, from which we have extracted the values comparable to ours (see table 2).

TABLE 2
PHILADELPHIA FEMALES 1910. COMPARISON OF THREE METHODS

AGE	PUB- LISHED	OUR METHOD		YULE'S METHOD		AGE	PUB- LISHED	KING'S METHOD ³	
	0c_x	0c_x	ERROR	0c_x	ERROR		0c_x	0c_x	ERROR
0	49.60	49.51	-0.09	49.87	0.27				
5	55.14	55.16	0.02	55.12	-0.02	7	53.75	53.75	0.00
10	51.24	51.23	-0.01	51.19	-0.05	12	49.48	49.51	0.03
15	46.83	46.84	0.01	46.79	-0.04	17	45.10	45.16	0.06
20	42.61	42.63	0.02	42.59	-0.02	22	41.03	41.08	0.05
25	38.72	38.74	0.02	38.69	-0.03	27	37.19	37.23	0.04
30	34.91	34.93	0.02	34.88	-0.03	32	33.39	33.45	0.06
35	31.15	31.18	0.03	31.13	-0.02	37	29.69	29.74	0.05
40	27.49	27.52	0.03	27.47	-0.02	42	26.02	26.08	0.06
45	23.82	23.85	0.03	23.80	-0.02	47	22.37	22.43	0.06
50	20.24	20.27	0.03	20.21	-0.03	52	18.87	18.93	0.06
55	16.89	16.93	0.04	16.87	-0.02	57	15.63	15.72	0.09
60	13.88	13.92	0.04	13.85	-0.03	62	12.79	12.86	0.07
65	11.25	11.29	0.04	11.22	-0.03	67	10.30	10.40	0.10
70	8.98	9.03	0.05	8.96	-0.02	72	8.13	8.21	0.08
75	6.93	7.03	0.10	6.90	-0.03	77	6.21	6.31	0.10
80	5.23	5.30	0.07	5.22	-0.01	82	4.64	4.74	0.10
85	3.81	3.91	0.10	3.84	0.03	87	3.32	3.43	0.11
90	2.69	2.80	0.11	2.81	0.12	92	2.34	2.29	-0.05
95	1.90	2.92	1.02	2.92	1.02	97	1.65	0.88	-0.77
100	1.32	3.00	1.68	3.00	1.68				

The marked error in the last two expectations in Yule's and our methods is accounted for by the fact that the observed $m_x^x + h$'s were biologically impossible, with $m_{100}^{\infty} < m_{95}^{99} < m_{90}^{94}$. The same error does not appear in King's method since the latter provides for rejection of these values, and extrapolation to obtain substitute values. Except for these terminal entries, our method is as good as the others, and much more rapid. (Yule's method is almost as rapid if sufficiently extensive exponential tables had been available, but logarithms had to be used.)

In Whipple's "Vital Statistics" it is shown that seven groups for adjusting death rates do as well for practical purposes as eleven groups.⁴ Presumably it should be possible to compute a skeleton life table including a figure for the expectation of life at birth on fewer age groups than we have just used without losing much accuracy and thereby to gain a great deal in ease of computation. Furthermore, in many cases of interest to

the health officer it is impossible to get enumerated populations or reported deaths except in fairly broad age groups so that if a life table is to be computed directly from the available data it must be of skeleton form.

Now what does happen when we use fewer groups? There are countless types of groups possible, but we shall consider those which we think would be apt to be used because of ready availability.

First, take the 13 groups used by the United States Census in publishing populations for moderately large places. The infants are kept separate from the children 1 to 4; there are five year groups from 5 to 35, and 10 year groups from 35 to 75 and all 75 and over are lumped into a final group [see table 3a].

TABLE 3
PHILADELPHIA FEMALES 1910. COMPARISON OF THREE GROUPINGS

AGE	PUB- LISHED $^o c_x$	3a OUR METHOD		3b OUR METHOD		3c OUR METHOD	
		$^o c_x$	ERROR	$^o c_x$	ERROR	$^o c_x$	ERROR
0	49.60	49.54	-0.06	49.61	+0.01	49.58	-0.02
1	55.28	55.16	-0.12			55.20	-0.08
5	55.14	55.13 ^a	-0.01	55.29	+0.15	55.17	+0.03
10	51.24	51.20	-0.04				
15	46.83	46.81	-0.02	46.96	+0.13		
20	42.61	42.60	-0.01			42.64	+0.03
25	38.72	38.71	-0.01	38.91	+0.19		
30	34.91	34.89	-0.02				
35	31.15	31.14	-0.01				
45	23.82	23.81	-0.01	24.08	+0.26	23.91	+0.09
55	16.89	16.88	-0.01				
65	11.25	11.28	+0.03	11.54	+0.29	11.28	+0.03
75	6.93	6.93	0.00			6.93	0.00

Our method fits slightly better than with the complete five year groups⁶ of table 2, no matter which of the three methods is used, suggesting that perhaps the grouping acts somewhat as a smoothing of the $m_x^x + h$'s. The assumption of a stationary population over 75 is not strictly valid; it gives the exact answer here, but that occurs rarely, and is not to be expected. A number of such calculations for different populations shows that there may be considerable error.

Second, for smaller places, the census often prints populations in six age groups beginning with "under 5," then two ten year groups, two 20 year groups and finishing with 65+ (see table 3b). Although the errors here are noticeable, still the results are probably good enough for practical purposes.

Third, we may give another grouping, with only 7 age groups, which may be made up from the 13 used above and which seem to have a some-

what greater biological or health interest than the second grouping (see table 3c).

We have used the enumerated populations and the reported deaths in certain age groups without smoothing or other adjustment. It is interesting to inquire what would be the result of applying our method to the age specific death rates taken out of the published life table. How well will the life table be reproduced? In five year groups, or the 13 census groups the reproduction of the table is excellent, though not notably better than when based on raw data. When done in the 6 census groups or 7 biological groups the errors seem to be considerably greater than for the computation as made directly from reported data. For other populations (than Philadelphia Females 1910) the results are similar and lead to the same conclusions, namely:

The short method of computation based on 13 census groups or the 7 so-called biological groups seems to give an entirely adequate life table for those entries computed, and the 6 census groups used for the smaller places will still give a tolerably satisfactory skeleton life table. While without a wide experience it would be impossible to tell under what circumstances, if any, the method would prove to give seriously incorrect results, a large experience indicates that the method can be recommended to health officers as likely to give sufficiently good results for their purposes, and is so short that the calculation can be made readily enough to make the life table technique almost as simple as that of adjusting death rates.

¹ "Some Life Table Approximations," by G. Udney Yule. *Proc. Internat. Math. Cong.*, held in Toronto, Aug. 11-16, 1924. Vol. II, p. 873.

² *Length of Life*, by Louis I. Dublin and Alfred J. Lotka, p. 312.

³ The figures for King's method as given here were the result of applying his method to the raw data—not the smoothed data which he recommends. Following his method rigidly gives a very good fit to published data, but the method was used on raw data for comparability with the other methods, and because a health officer desiring a short method would certainly be applying it to raw data.

⁴ *Vital Statistics*, by George C. Whipple, p. 297.

⁵ A trial of Yule's method for the groupings of table 3 indicates that it does not give such good results as ours, besides being somewhat slower in computation. We shall therefore omit further comparisons with it.

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

October 15, 1938

Number 10

Copyright 1938 by The National Academy of Sciences

*PRELIMINARY REPORT ON RECENT GEOLOGICAL AND
ARCHAEOLOGICAL DISCOVERIES RELATING TO EARLY MAN
IN SOUTHEAST ASIA*

BY H. DETERRA*

ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA

Communicated September 13, 1938

The Ice Age, which was a principal determinant of human prehistory, is very little known in Southern and Eastern Asia; in fact glacial cycles have never been established east of the northwestern Himalayas. However, in India, China and Java there are abundant data proving that this region was important during the Old Stone Age. The variety of evidence for a center of human origins in Asia called for the investigation of an area intermediate between the known regions in India and the Far East. Burma was selected, since there the geology of the Cenozoic has much in common with that of India and China. In addition, the pioneer work of Mr. T. O. Morris had already provided some information on terraces and Stone Age cultures along the Irrawaddy River. In Burma also, I could continue my own work on the Pleistocene of India, the results of which are at present being published by the Carnegie Institution of Washington.

In this undertaking my colleague, Dr. Teilhard de Chardin, offered me his coöperation, and in addition the Peabody Museum of Harvard University delegated Dr. H. L. Movius as archaeologist to the party. This expedition, jointly organized by the Academy of Natural Sciences of Philadelphia and the Peabody Museum of Harvard University with the generous assistance of the American Philosophical Society, has just returned from Burma and Java, and in the following report a brief summary is given of our studies as far as they can be evaluated at the moment.¹

THE ICE AGE IN UPPER BURMA.—The region investigated comprises an area roughly 200 miles long and some 50 to 150 miles broad, extending from the Irrawaddy lowlands of the oilfield region, northeastward as far as the Salween River on the Chinese border. Flanked in the west by an anticlinorium of the Arakan Yoma Mountains and to the east by the Shan Plateau, the Irrawaddy Basin makes a geosyncline in which over 50,000

feet of Tertiary rocks have been deposited. Through this trough the Irrawaddy River discharges annually some 236 million tons of silt, which is dumped into a delta that advanced from 3-4 miles in the last century toward the Indian Ocean. In the lower 450 miles of its course, between Mandalay and the delta, the stream is well graded, but during the Quaternary it experienced repeatedly changes of level, as recorded by the terraces which we studied, mainly between Magwe and Mandalay.

In *The Terrace System of the Irrawaddy Valley*, we recognized the following most salient features:

1. The terraces are superimposed on the *Irrawaddy Series*, a folded and peneplained river formation, the upper portion of which contains a mammal fauna analogous to that of the *Upper Siwaliks* of India. These belong to what Dr. Teilhard has called the *Villafranchian* fauna of Eurasia.

2. Occupying an old valley, the terraces are composed of coarse, boulder-bearing gravels of red color, which mark three different stages of aggradation.

The highest terrace gravel is preserved by a group of isolated hills, situated near the oilfield of Chauk, some 300 feet above the stream. The size of the pebbles, 5 to 10 inches in diameter, depicts an ancestral Irrawaddy much more powerful than the present river. A lateritic soil mantle in the adjoining highland appears to be connected with this stage, which was clearly a period of greater rainfall. (At present this region belongs to the Dry Zone of Burma with 60 to 70 inches of annual rainfall; this occurs almost entirely from June to September. No lateritic soils are formed here at present.) At one place the highest terrace was found to contain a few flaked pebbles of fossil wood and silicified tuff, which may be artificial. This terrace appears to be strongly tilted.

The second and third terraces lie 180 and 100 feet, respectively, above river level and are associated with a thick series composed of red gravel and sand. In it a Middle Pleistocene type of fauna was found at Mingun, opposite Mandalay (see *Palaeontological Report*), and further downstream an Early Palaeolithic industry, called *Early Anyathian* by Dr. Movius (see *Archaeological Report*). A prolonged interval of erosion and aridity preceded the deposition of the second river drift, and then a valley with ferruginous soil caps, containing the earliest prehistoric industry, was formed. The second river gravel buried these caps, but the basal layers of gravel are rich in rolled Early Palaeolithic tools. Again this was a time of greater rainfall, as indicated by thick fans of *Red Earth* which were released from the highlands at this stage.

The fourth terrace is composed of a third type of gravel, which is generally less coarse and more sandy. A somewhat advanced type of Palaeolithic was found in this deposit. The fifth terrace approaches the recent river deposits in composition, and it may be post-Pleistocene in age.

3. The terraces are associated with soils corresponding to periods of greater and lesser rainfall. The first and second types of the old gravels, underlying Terraces I-III, are dominantly reddish; they are connected with lateritic soils on the adjoining land-surfaces. From here *Red Earth* was twice washed into the valley, a process which is made especially clear by the composition of the second terrace; the fine sand strata of which merge laterally into thick lateritic slopewash deposits. Increasing aridity is indicated by the formation of loessic sediments during the fourth terrace stage. This soil is yellow to pink in color, and mainly structureless. It has drifted in the manner of true loess up to the third terrace, the erosional surface of which was thus again buried. The composition of the fourth terrace, however, proves that this represents a major and prolonged fill stage, presumably again in the nature of a *Pluvial Period*.

4. The Irrawaddy terrace system is of regional extent, since it was found also in the adjoining Shan Plateau in the reaches of the Namtu and Salween rivers.

The interpretation of these features leads to conclusions which are of import both to the geologist and archaeologist. In the first place it is a fact that this terrace system of Upper Burma resembles closely the one found in the Siwalik region of Northern India (see my preliminary report, *Science*, 1936, No. 2149). The same number of terraces, a similar succession of aggradational and degradational stages, and an archaeological succession of prehistoric cultures in which Early Palaeolithic core-pebble tools are gradually mixed and partly replaced by flake tools of a more advanced type, are all present. In both regions the terrace formation began in the Middle Pleistocene, probably as a result of the relative geological stability then established in the Himalayan forelands, where the Early Pleistocene beds are all strongly folded.

In the memoir mentioned above, it will be shown that the terrace system of Northwest India reflects both climatic and diastrophic processes. In the latter region, Terraces II and IV represent the third and fourth Himalayan glaciations. Loessic soils are associated with them, and indications of pluvial conditions are to be traced directly to corresponding periods of glaciations. Whereas in India these terraces were found some 150 miles away from the respective moraines of the third and fourth glaciers, in Burma they are clearly recorded 350 to 400 miles distant from the southernmost limit of the ice-sheets. Considering the sedimentary records, as previously outlined, we believe that they are not merely the result of climatic cycles restricted to the respective highlands, but that they can be understood only as *Pluvial* and *Interpluvial* stages corresponding more or less to *Glacials* and *Interglacials*.

On geographical and climatic grounds, it can be demonstrated that the

Quaternary stages of India and Burma were similarly recorded in other regions, as for instance in China.

It will be noted that the Early Pleistocene has thus far not been classified into stages to the same extent as the Middle and Late Pleistocene. The reason is, as has already been emphasized, that they are hidden in a more compact pile of folded strata which do not readily permit of the same detailed analysis. However, in this respect the older Pleistocene beds of Burma have yielded certain palaeontological data which helps to fix the stratigraphic position of the *Upper Irrawaddy Series*.

PALAEONTOLOGY OF THE PLEISTOCENE IN BURMA.—The *Upper Irrawaddy* fauna has been known for some time, especially through studies by Noetting and Pilgrim; lately Colbert has analyzed it anew with Dr. B. Brown's collection. The vertebrate remains collected by us can only confirm the conclusions drawn by previous investigators who emphasized the Indian, specifically the *Upper Siwalik*, type of fauna, represented in this series. In our material forms such as *Leptobos*, *Bubalus*, *Stegodon*, *Elephas planifrons* and *Equus* are represented, together with types of lesser stratigraphic significance. In view of the existing differences of opinion concerning the age of the *Upper Siwalik* fauna (Late Pliocene or Early Pleistocene), it is interesting to observe that the *Upper Irrawaddy Beds* have yielded for the first time invertebrate and plant fossils. The state of their fossilization, and the type of freshwater fauna represented, clearly indicate a Pleistocene age, at least as far as the upper 1000 feet of this series is concerned. Moreover, the ancient terrace gravels overlying these beds contain a Middle Pleistocene type of fauna (*Elephas namadicus*, *Bos* cf. *namadicus*, *Hippopotamus*), reminiscent of the *Narbada* Pleistocene series of peninsular India, and such a succession would be difficult to understand if the underlying beds were to represent the Late Pliocene.

Of special interest was the discovery of fossiliferous fissure deposits in the Shan Plateau, near Mogok. With the exception of a skull of *Ailoropus* nothing was known of the nature of this fauna. It throws a new light on former faunistic relationships of the Indian with the Chinese mammal world. The presence of *Stegodon*, *Elephas namadicus*, *Bos*, *Rhinoceros*, deer and porcupine links this fauna with that characteristic of the fissures found in the neighboring provinces of South China.

CORRELATIONS WITH SOUTH CHINA.—The conclusions arrived at by Dr. Teilhard de Chardin with regard to existing analogies between the Late Cenozoic of Burma and China can be only mentioned very briefly in this report. He points out that such analogies are striking "mainly: (a) in the Late Pliocene lake-deposits; (b) in the Early and Middle Pleistocene gravel formations; (c) in the fissure deposits. All three are practically con-

tinuous between Eastern Burma and the Yangtse Basin. They must therefore be contemporary, and express the same series of diastrophic and climatic changes over the same geological unit." Hence, the terraces of the Yangtse may be linked to those found in India and Burma, and the "possibilities of covering under a single stratigraphic and physiographic scheme the Late Cenozoic history of the whole South and Central Asiatic land-mass becomes now an assured probability."

STONE AGE ARCHAEOLOGY OF BURMA.—Dr. Hallam L. Movius reports about his archaeological work as follows: "Burma has contributed a new and important link in our existing knowledge concerning the development of the Stone Age in Southeast Asia. In the Middle Pleistocene gravels of the Irrawaddy Valley, especially at Yenangyaung, Chauk and Nyaungoo (near Pagan), fairly extensive remains of a new Lower Palaeolithic culture were discovered by the Joint American Expedition for Early Man. This culture, to which the name *Anyathian* has been applied, seems to form a break between the Palaeolithic of Southern India and that of the Far East, since in it fist-axes are completely absent. The implement types—choppers, crude scrapers, flakes and cores—have exact typological parallels in the *Patjitanian* of Java, discovered by Dr. von Koenigswald. In the latter region, however, fist-axes are present. But the similarities of the two cultures in all other respects suggest that both developments may have spread from a common source, perhaps in South China. On the other hand the varied assortment of tools found in Java may indicate that the center of this development is to be sought further to the south.

"The Anyathian implements are for the most part heavily rolled and patinated. They are made either of silicified tuff or fossil wood. The Early Anyathian (Lower Palaeolithic) occurs *in situ* in the basal gravels of the third terrace of the Irrawaddy Valley which contain the remains of *Elephas namadicus*. The forms are heavy and crude; core implements predominate. A later development of this culture (perhaps influenced by an Upper Palaeolithic center outside the region) is represented by the Late Anyathian. This occurs on the surface of Terrace III and *in situ* in Terrace IV. Essentially the same tool forms persist, but there is a marked tendency to specialization, and small implements are characteristic. No trace of a transitional, or Mesolithic, stage between the old and the New Stone Age was discovered. Neolithic material was found over a wide area in Upper Burma associated with polished stone axes and pottery.

"As Dr. deTerra points out, the Stone Age chronology established for Burma is substantiated by stratigraphic records. At present many archaeological gaps still exist, but the main development is clear. It seems apparent, however, after a preliminary study of the material collected in

the field, that in both Burma and Java, we are dealing with a new, Far Eastern, centre of Lower Palaeolithic development to which the long established European classification cannot be applied. Furthermore, the strong negative evidence regarding the absence of fist-axes in Burma makes it seem very unlikely that the material discovered in Java owes its inspiration to the Lower Palaeolithic of India. However, the Soan, which appears to be an early intrusive element in Northwestern India, was apparently derived from the same Far Eastern source, but with this exception, direct Indian parallels are absent."

EXCURSION TO JAVA.—Our field season in Burma closed at the end of March when all members of the expedition proceeded to Java. Here, we visited, under the expert guidance of Dr. G. H. R. von Koenigswald, the most important places where either fossil man or Old Stone Age cultures have been discovered in recent years. In this paper it is impossible to do full justice to the truly remarkable wealth of information which Java holds with regard to Quaternary geology and Early Man.

Particularly impressive to us was the Solo Valley with its terraces containing Upper Palaeolithic material and the skulls of *Homo neanderthalensis soloensis* (Oppenoorth). One cannot help but feel that a detailed physiographic survey of this region would furnish a key to a more detailed stratigraphy, which thus far has been founded mainly on palaeontological data. In this region it became evident that the Quaternary cycles in Java differ in many respects from those found in continental Asia. For one thing, in Java volcanism has introduced processes of sedimentation whose cyclic character is not readily recognized. Also, the climatic records of the humid tropics differ altogether from those found in the more arid northern latitudes, and the effect upon fauna and sediments is such as to make direct correlations with the Quaternary of the Asiatic mainland less apparent than was at first thought to be the case.

The new site of the *Pithecanthropus* skull and mandible near Sangiran, north of Solo, assures beyond doubt the Middle Pleistocene age of this fossil. Its stratigraphic location was in the lower portion of the *Trinil Beds* which are overlain by some 150 feet of Middle and Upper Pleistocene fossiliferous strata, all clearly exposed in one section. Especially clear is the position of the infant skull of *Homo modjokertensis* (v. Koenigswald), near Modjokerto, in Eastern Java. Despite the relatively shallow depth at which the skull was discovered (3 feet), it was evident that in the absence of soils and terraces, nothing could have obscured the true location and stratigraphy of this site. Its age is, according to Dr. von Koenigswald, Lower Pleistocene because of its association with such mammal forms as *Hippopotamus antiquus* and *Cervus swaani*, which appear to be ancestral to later forms found in the Trinil horizon.

ACKNOWLEDGMENTS.—In concluding this report, I wish to express my sincere appreciation for the financial support which has enabled me and my associates to carry out this work. Acknowledgment is especially due The American Philosophical Society, Harvard University and The Carnegie Institution of Washington in this regard.

I wish also to thank the Director of the Geological Survey of India, Dr. A. M. Heron, the members of the Geological Department of Burma and our colleagues in Java for the friendly coöperation extended to us.

* Field Director of the American Southeast Asiatic Expedition, 1937-1938.

¹ A short account of the results of this expedition has already appeared, see deTerra, H.; de Chardin, P. Teilhard; Movius, H. L., *Nature* 142 (1938).

THE TIME COURSE OF PHOTOSYNTHESIS AS SHOWN BY THE GLASS ELECTRODE, WITH ANOMALIES IN THE ACIDITY CHANGES*

BY L. R. BLINKS AND R. K. SKOW

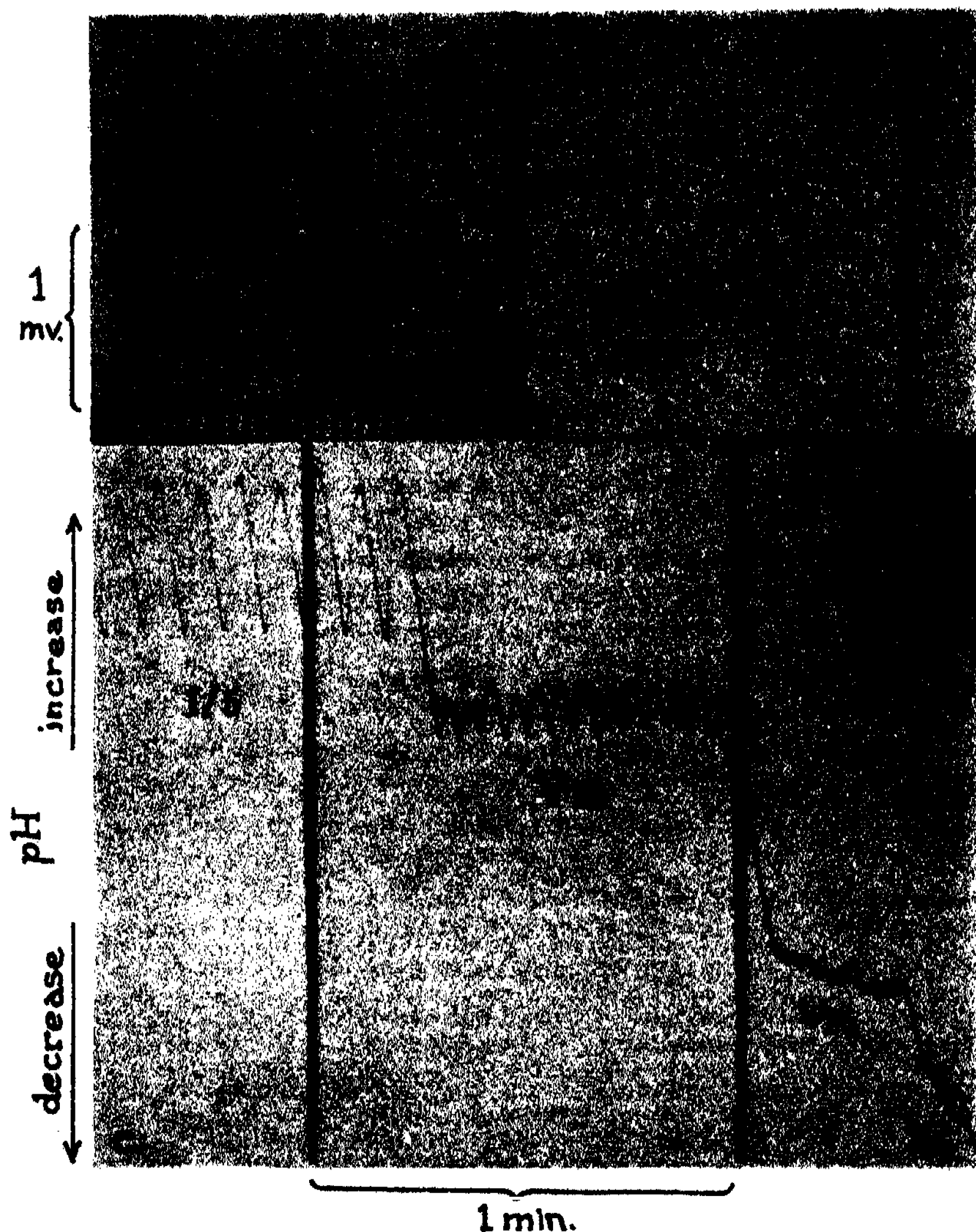
JACQUES LOEB LABORATORY, HOPKINS MARINE STATION, AND THE SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated Aug. 26, 1938

Many features of the course of bio-electric potential change in plant cells during illumination¹ are best explained by the acidity changes resulting from CO₂ assimilation. However, certain cusps during the first moments of illumination (as well as of darkening) suggested to us that there were unsuspected anomalies, or even reversals, of the normally expected drifts. Since these might be complications of the bio-electric rather than of the photosynthetic mechanism, it was desirable to test the time course of acidity change by independent means. An appreciable volume of solution being required for indicator methods,² the authors have decreased diffusion time as much as possible by applying a glass electrode in direct contact with the tissue, or layer of cells settled from a suspension.³ This reduces the diffusion distance (from chloroplast to electrode) to 2 or 3 microns in favorable cases, and keeps the volume of solution restricted to a very thin film. Consequently pH changes due to CO₂ assimilation or production are very prompt. The time curves, being the most rapid yet obtained for the course of photosynthesis, may be of interest beyond the problem which inspired them.

The speed and sensitivity of the method are illustrated in figure 1a, which shows the response to an intermittent light, flashing 34 times per minute,

* Aided by a grant from the Rockefeller Foundation.



FIGURE

Photographic records of the deflections of a d'Arsonval galvanometer (period 1.5 seconds) balanced into the plate circuit of a vacuum-tube amplifier with grid connected to glass electrode (MacInnes glass: Corning 015). The electrode was a thin walled, flat bottomed bulb, containing a thick suspension of motile cells of the unicellular marine alga *Stephanoptera*. Some of the cells had settled to a layer on the bottom. Illuminated with incandescent lamp (6000 meter candles); infra-red largely absorbed by CuSO_4 filter. In *a* the light was interrupted by a rotating sector making a revolution every 1.75 seconds; light period $\frac{1}{4}$ of this time or 0.44 sec.; dark period 1.32 seconds (period shown by black stripes at the bottom of *a*). In *b*, 11 flashes were given by camera shutter, lasting $\frac{1}{2}$ second each, about 5 seconds apart. In *c*, shutter flashes of $\frac{1}{4}$, $\frac{1}{10}$, $\frac{1}{32}$, and $\frac{1}{16}$ seconds were given, at inter-

the light flashes being $1/4$ as long as the dark periods. Neither diffusion mixing nor galvanometer period wholly obscures the external effects of the changes in the cell, which must pass out in waves which are not wholly fused or smoothed. Indeed, responses are obtained from much briefer illuminations, such as $1/8$, $1/10$ and $1/36$ second, as shown in figure 1c. $1/80$ -second flashes of this intensity give a just perceptible response, which with increased light intensity becomes well marked.

Wider separation of the briefer flashes shows that the perceptible response is in the opposite direction to that found during longer illumination (Fig. 2). The pH *decreases*, the acidity increases, as the result of this brief illumination. Even with $1/10$ -sec. flashes, the first effect is in the same direction, although in later and longer flashes this tends to be swamped out by the following wave of alkalinity. However, if the flashes are given after a considerable dark period (Fig. 2b), the acid gush is much more marked with brief flashes, and remains as an initial downward cusp in the alkaline wave produced by $1/8$ -sec. illuminations; even at $1/2$ sec. there is a slight initial downward (acid) movement.

With very much longer light and dark periods, some of the same effects persist. Figure 3a shows 2 light flashes of 8 seconds, with dark periods of about 40 seconds. The steep rise of pH in the light, and slower fall in the dark are very striking, and exactly what would be expected. Since the movement is so fast, the lag at the onset of a light or dark period is partly due to inertia of the galvanometer, and the continuing powerful diffusion wave from the cells, which may obscure the anomalies, even if present in this mid-pH range. They are better shown when the system has reached its light and dark asymptotes (when CO_2 diffusion equilibrium is reached with the overlying solution). Thus in figure 3b, after a long dark period, illumination produces an acid gush that lasts 5 or 6 seconds before the alkaline drift predominates; and on darkening there is a marked cusp in the opposite direction, with a later hump before the slower acidity drift of respiration sets in. Similarly in c, after a long period of light, darkening produces an extreme cusp in the opposite direction: an increase of alkalinity before the acid drift of respiration sets in. (A second light and dark period, following, now resemble figure 3a, with galvanometer over-shooting obscuring possible anomalies.)

Sometimes the acid gush is delayed, so that it takes the form of a re-

FIGURE 1 (Continued)

vals corresponding to the galvanometer deflections (indicated by marks for the $1/80$ -second exposures).

The sensitivity is shown in a, 1 mv. corresponding to about 0.017 pH unit. (The perceptible response to a $1/80$ -second flash is thus about 0.001 pH unit.) pH increase (alkalinity) is upward on the scale; pH decrease (acidity) downward. Large time marks 1 minute apart, as indicated on c.

cession in the alkaline drift, which starts promptly, then falls, and rises again. A similar notch in the curve often occurs on darkening. These would be more uncertain of interpretation if clear cases of acid gush like figure 3 were not common.

Not only may the acid gush be obtained with a variety of marine algae, but with fresh water and land plant leaves. An example with a floating leaf (water lily) is shown in figure 4. Equally good records have been obtained with submerged leaves (*Potamogeton*), and with land plants (castor-bean, corn). In these also the acid gush is most marked on illuminating

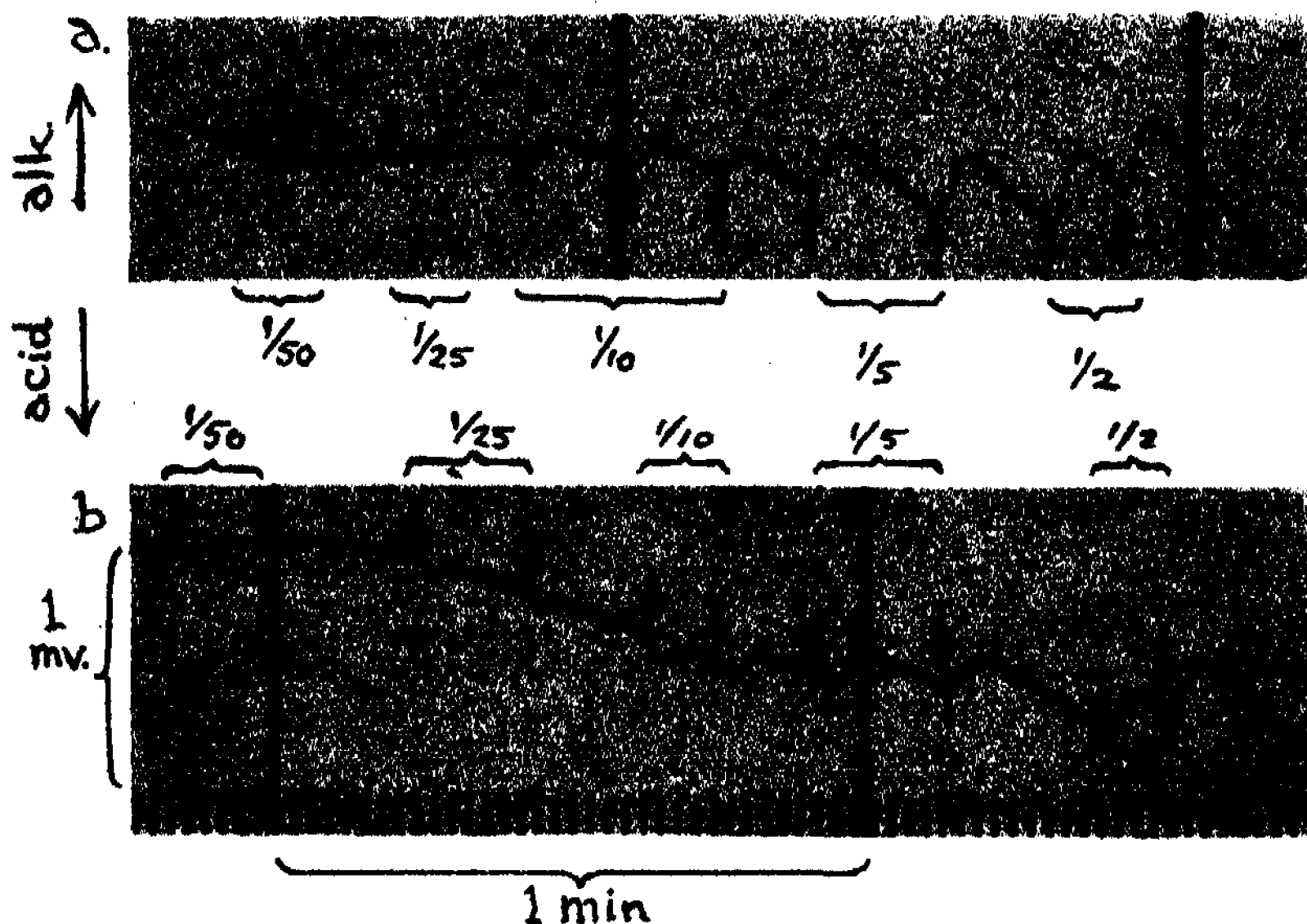


FIGURE 2

Stephanoptera suspension in glass electrode, as in figure 1. Incandescent light (6000 M. C.) given in flashes of fractions of a second, as marked, by camera shutter. In *a* after a short dark period, in *b* after a long dark period. Beginning of illumination period indicated by the arrows. Large time marks, 1 minute apart. Small marks at base of *b* are 1.75 sec. apart.

after long dark periods, and the alkaline gush on darkening after long light exposures, before the regular drift sets in. If stomata are closed, or if the surface has no stomata, light has practically no effect. Albino leaves (e.g., genetically white corn seedlings) also show no response to light, although their Mendelian green dominants give good responses, including the anomalies. Non-photosynthetic plants, such as yeast suspension, also show absolutely no light effect. A general protoplasmic response, such as altered permeability with release of acids or bases, is thus ruled out.

Ammonia, which makes bio-electrically evident the anomalies in *Halicy-*

is, has little or no effect upon the glass electrode response. Cyanide and urethane greatly reduce, or entirely abolish the normal response, although in lower concentrations they sometimes exaggerate the anomalies (this may give a clue to their mechanism). Photosynthetically active regions of the spectrum give the best pH changes, including the anomalies. Some connection with photosynthesis seems indicated, but it is too early to estimate the rôle or importance of the anomalies. The following possibilities may be considered, however:

A. Release of CO_2 by some photolabile holding mechanism.⁴

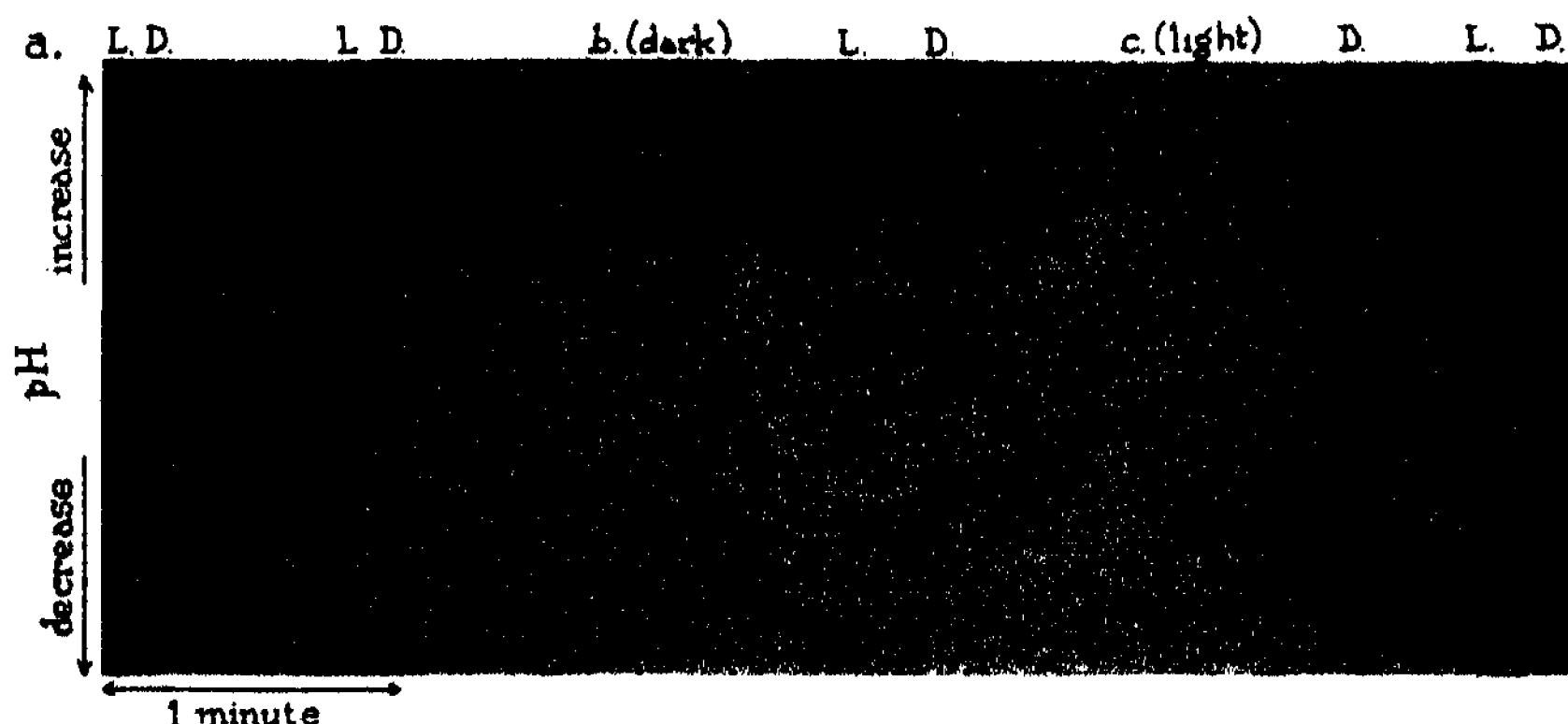


FIGURE 3

Stephanoptera suspension in glass electrode, as in figure 1. In *a*, 2 light flashes (6000 M. C.) of 8 seconds each are given (L for light, D for dark). This is in the middle of the pH range between light and dark asymptotes. In *b*, light is given at the end of a long dark period when respiration has carried the pH close to the acid limit (most of the previous drift having been compensated to keep the image on the record). An increased acidity is produced by light before the alkaline drift sets in. (Note cusp on darkening.) Record *c* was taken after a long light period, when the pH (also compensated) had been raised by photosynthesis to its upper limit (most of the CO_2 removed from solution). There is an "alkaline gush" on darkening, preceding the acidity drift due to respiration. A second light and dark period in *c* resemble *a*.

Direction of pH change indicated at the left, sensitivity about as in figure 1. One minute time interval shown at base.

B. Increased respiration, stimulated by light in excess of photosynthesis. (This might result from the initial oxidation of metabolites with an R. Q. larger than 1.0.⁵)

C. Production of an acid (stronger than carbonic) as the first product of photosynthesis.

D. Photo-decomposition of compounds (e.g., malic acid to CO_2 which then diffuses out of the cell. Suggestion of Dr. H. A. Spoehr).

E. Increased consumption of a base, e.g., ammonia during the first moments of illumination.

The converse of these would account for the alkaline gush on darkening.

It is clear that the glass electrode is unable to distinguish between carbonic and other acids. But since carbonates are almost universally present in and around cells, increased acidity due to any acid would release CO_2 ; its increase might give a momentarily higher photosynthetic rate, if CO_2 happened to be a limiting factor. This might account for increased efficiency in flashing light under such conditions, and an actual increase in O_2 production is often found during the first moments of illumination.⁶

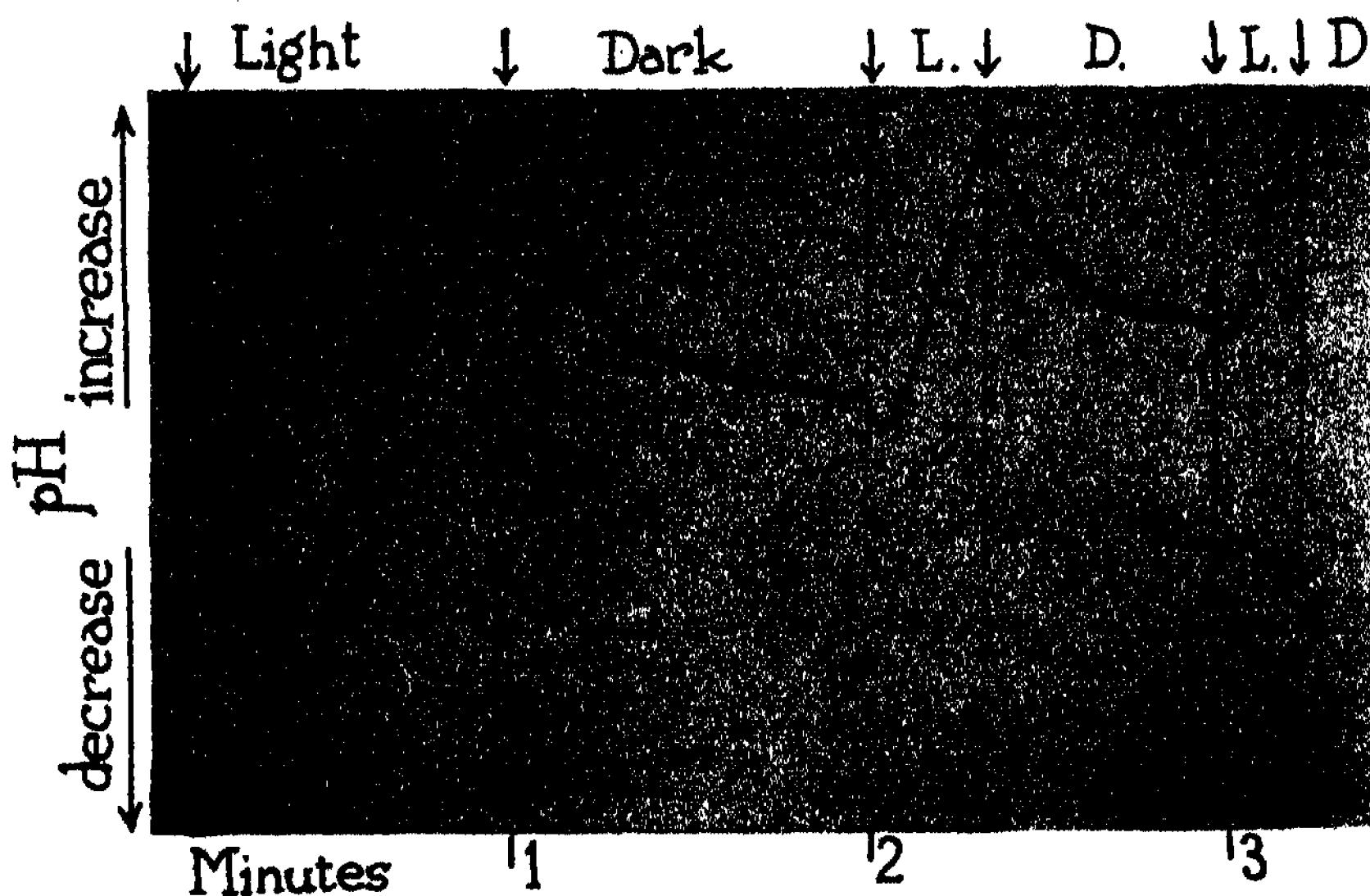


FIGURE 4

Acidity change on illumination as shown by glass electrode closely pressed against the upper surface of floating leaf of pond lily (*Castalia*). (This side has stomata, the under side has none.) A temporary decrease of pH ("acid gush") occurs immediately on illumination, preceding the regular rise of pH (alkalinity increase) due to assimilation of CO_2 . The anomalous pH decrease is most marked on the first illumination (after a long dark period) and becomes progressively less on successive exposures after shorter dark intervals. There are no anomalies on darkening after these short light periods, but these appear after long light periods (cf. figure 3c).

Light intensity about 6000 M. C. (CuSO_4 filter). Direction of pH change shown at left, with time in minutes at base. pH sensitivity about as in figure 1.

It is also clear that the effects within the cell itself might well be much more intense than we can pick up outside. For example, if a strong acid were released internally, it might decompose the available carbonate, inside the cell, releasing a small amount of CO_2 after which no further acidity would be noted outside, since the strong acid could not come out of the cell. This may be why the anomaly appears best after a dark period, when the

carbonates could again be built up inside the cell. Certainly the bio-electric anomaly often lasts longer than the acidity change which the outside electrode can show.

Another shortcoming of the closely appressed glass electrode is that it cannot show absolute rates without many corrections for diffusion, altered buffering, etc. But it has its value in indicating the direction, and the relative speed of photosynthesis during the first moments of illumination. It is believed that the anomalies are not artifacts, since obvious controls with dead tissue (boiled), green cellophane, black paper, agar or the blank electrode, failed to show any significant response to light. Infra-red was largely filtered out; when used alone it gave only a slow drift due to heating, quite different from the cusps in the visible light. More rapid recording instruments, such as the string galvanometer and cathode ray oscillograph, will be applied to show the diffusion lag, induction period and the response to very brief flashes.

An analogous rapid electrode method for oxygen changes has been developed and will be described in another communication.⁵

¹ Experiments on *Halicystis*, *Nitella* and *Valonia*, to be published shortly. See also Marsh, G., *Carnegie Inst. Washington Year Book*, 36, 99 (1936-1937).

² Osterhout, W. J. V., and Haas, A. R. C., *Jour. Gen. Physiol.*, 1, 1 (1918-1919).

³ While the first trials of the glass electrode method were being made in this laboratory (by Mr. Fred L. Kirby, 1936-1937), an analogous application for muscular pH changes was reported (Dubuisson, M., *Arch. Ges. Physiol.*, 239, 314 (1937). Cf. also Maison, G. L., Ort, O. S., and Lemmer, K. E., *Am. Jour. Physiol.*, 121, 311 (1938). A glass electrode has also been employed in a large volume of suspension of *Chlorella* cells (Tang, P. S., and Lin, C. Y., *Jour. Cell. Comp. Physiol.*, 9, 149 (1936-1937). But here the solution was well buffered, since the purpose was to avoid, rather than to obtain pH changes, during oxidation-reduction potential measurements. Nevertheless a small anomalous pH change occurred.

⁴ Shafer, J., *Plant Physiol.*, 13, 141 (1938).

⁵ Blinks, L. R., and Skow, R. K., *Proc. Nat. Acad. Sci.*, 24, 420-427 (1938).

THE TIME COURSE OF PHOTOSYNTHESIS AS SHOWN BY A RAPID ELECTRODE METHOD FOR OXYGEN*

By L. R. BLINKS AND R. K. SKOW

JACQUES LOEB LABORATORY, HOPKINS MARINE STATION, AND THE SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated September 7, 1938

The application of a glass electrode in direct contact with plant cells or tissues¹ proved so valuable in following rapid CO₂ changes during and after illumination, that a corresponding method for following oxygen evolution and consumption was desirable. While the well-known leuco-base and luminous bacteria methods² are extremely sensitive, they are useful in only a limited range of oxygen tension, close to anaerobic conditions, where they respond maximally to slight oxygen increases, after which no time curve can be obtained. Similar objections would hold for an electrical indication of the same effect, namely, a bright platinum electrode with an added redox system (such as methylene blue) which would become reduced in the dark by respiration, and oxidized by evolved oxygen in the light, with corresponding potential changes.³

A much broader oxygen tension range is covered by the dropping mercury cathode ("polarograph") polarized at a potential to reduce oxygen; this is the Vitek method⁴ for determining dissolved oxygen, which has been applied to physiological problems for several years,⁵ including a recent extension to photosynthesis.⁶ The authors have employed this method for photosynthesis, but found an appreciable time lag between illumination and the electrode response, due to the volume of solution necessary to give space for the mercury drops to form and fall without hindrance, close contact with the tissue being impossible. The oscillations of current produced by the drops would also obscure any very rapid changes of oxygen evolution (Fig. 1a).

A stationary electrode was therefore employed, in direct contact with the tissue, reducing the diffusion distance to the thickness of the cell wall and a very thin film of solution—probably not over 2 or 3 microns total distance. By analogy with the dropping cathode, the electrode was first of mercury, which has the advantage of making a very tight fit with irregular surfaces. But the danger of poisoning the tissue, even with the cathodically reduced mercury surface, led to the substitution of bright platinum, with identical results, and greater assurance against biological alterations.

The principle is the same as that of the dropping cathode, except that the surface not being constantly renewed, becomes steadily polarized with much less residual current density; a larger surface compensates for this,

* Aided by a grant from the Rockefeller Foundation.

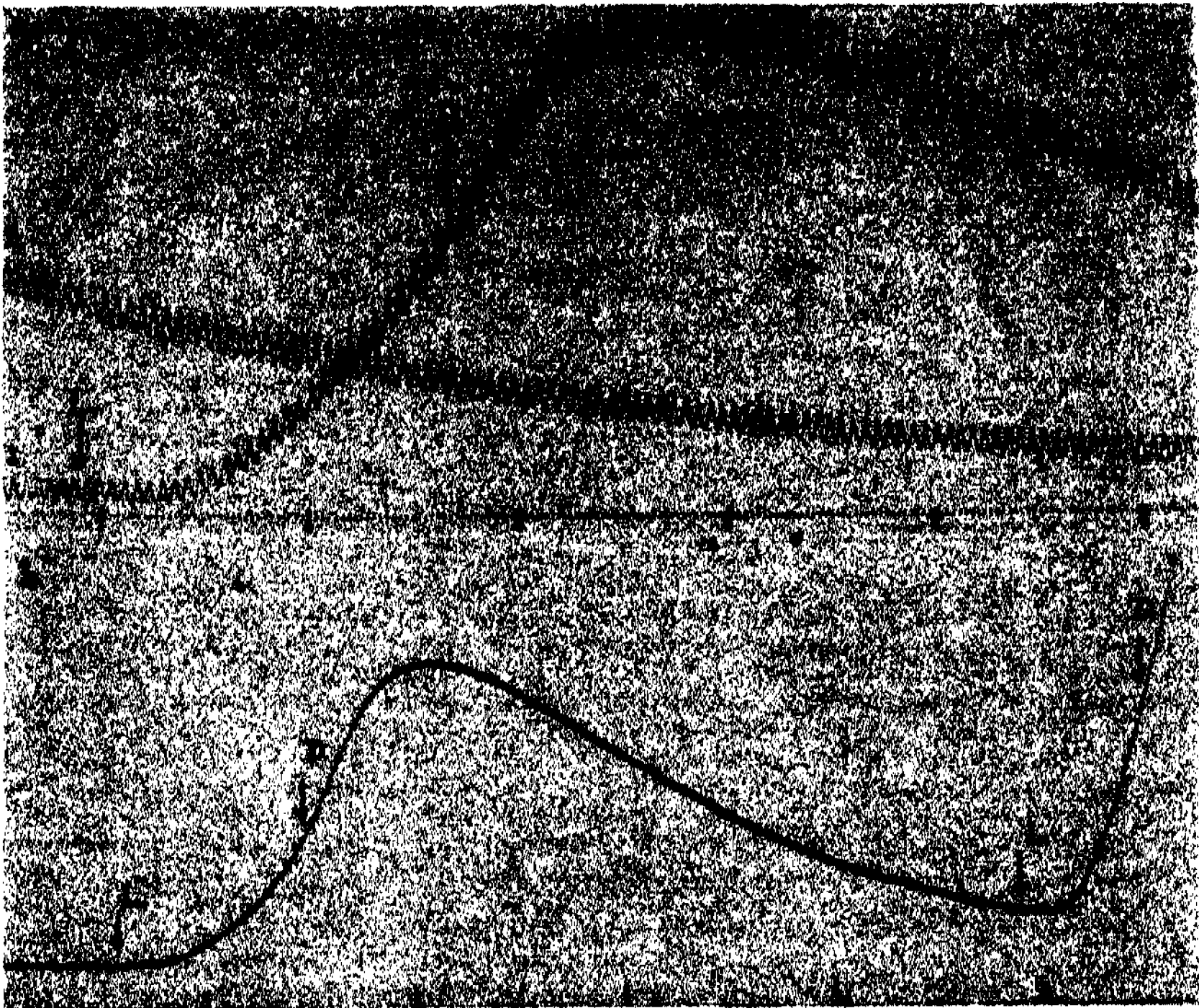


FIGURE 1

Galvanometer records showing use of electrodes polarized cathodally at 0.5 volt for indicating oxygen changes during photosynthesis and respiration. A small glass tube was lined with a piece of the marine alga *Ulva*, and filled with sea water into which dipped the electrode. In *a*, this was a dropping mercury cathode, brought as close to the tissue as possible; in *b* it was a coiled platinum wire at the same distance. A salt bridge connected these to a calomel electrode as anode, with galvanometer and potential source in series. The full height of record *a* represents about half the O_2 content of sea water in equilibrium with air; the sensitivity in *b* is considerably greater. The oscillations in *a* represent the dropping rate of the mercury.

The O_2 content had fallen to a low and nearly constant value at the beginning of each record. At *L* the plant was illuminated with an incandescent light (6000 M. C., with $CuSO_4$ filter for infra-red). After a delay of 10 or 15 seconds, caused by diffusion from tissue to electrode, curves rose rapidly due to O_2 evolution in photosynthesis. At *D* the plant was darkened, and, again after a diffusion lag, the O_2 content slowly fell, due to respiration plus reduction by the electrodes. In *a*, this fall is continued on the second revolution of the drum (1 minute being omitted between records 1 and 2). In *b*, a second illumination (*L*) caused a steep rise in O_2 content, but again with lags at illumination and darkening. Large time marks at base of records 1 minute apart. (Actual photographic records of galvanometer deflections, the fine lines of *a* being traced with India ink for contrast.)

however. The residual current represents a steady state set up between the reduction of oxygen at the electrode and its diffusion from the body of

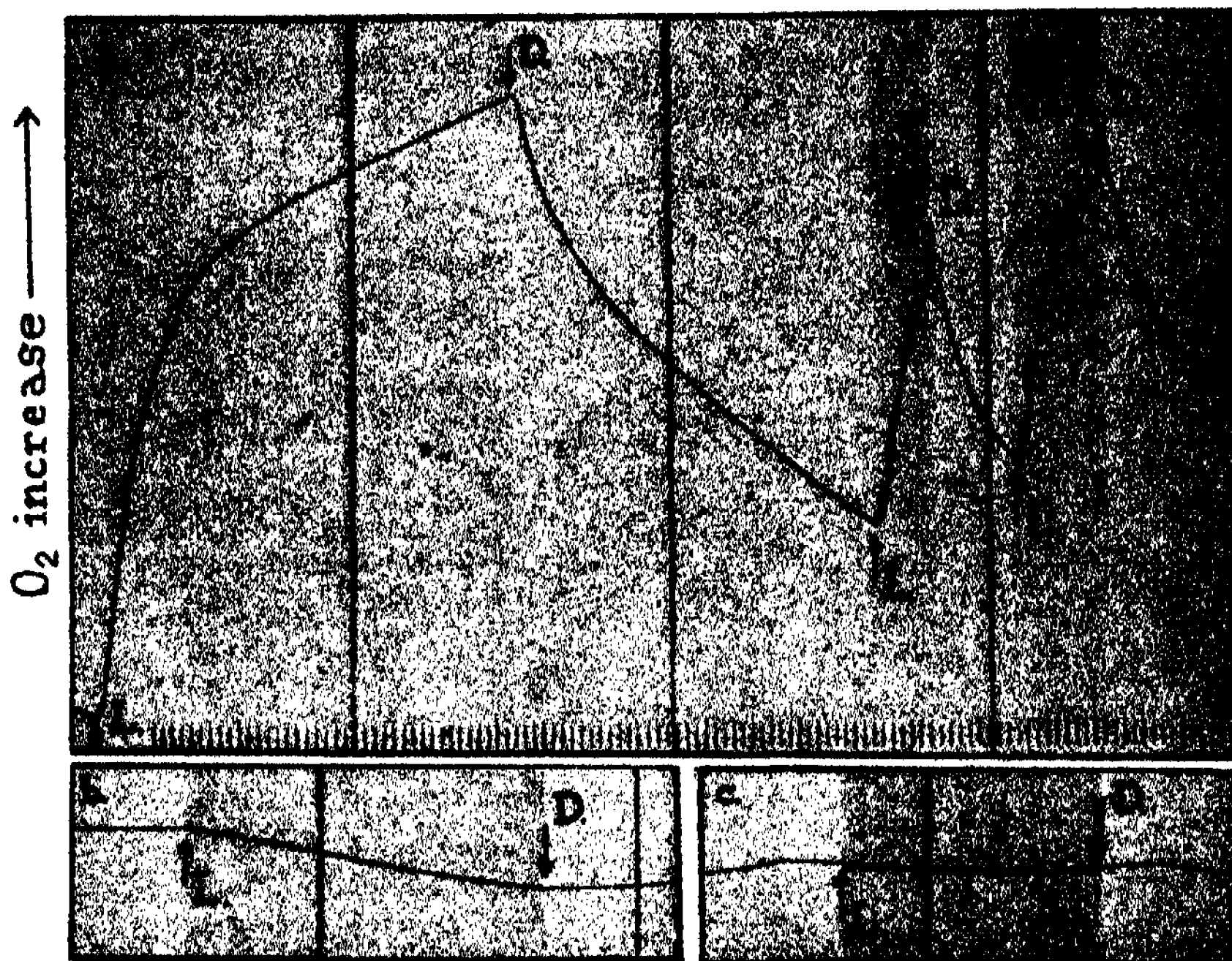


FIGURE 2

Deflections of galvanometer in series with stationary platinum electrode, polarized cathodally at 0.5 volt. In *a* a piece of living *Ulva* thallus was pushed tightly against the surface by means of a thin agar disc, and the O_2 in the intervening sea water film had dropped to a low, but not fully anaerobic level. At *L* the *Ulva* was illuminated through the agar, giving an instant increase in O_2 content by photosynthesis. At *D* the thallus was darkened, showing the disappearance of O_2 by respiration plus electrode reduction. Two shorter light and dark periods follow.

In *b* a piece of dead *Ulva* (previously boiled) was similarly exposed, and in *c* the electrode alone; the slight downward drift is probably due to warming with increased reduction of O_2 at the electrode. Equal absence of effect is shown by a living tissue between two agar blocks with the current flowing, indicating that changes of resistance in the tissue are not responsible for the effects.

Actual photographic records of galvanometer response, the fine lines in *a* traced with India ink for contrast. Sensitivity the same in all, the rise in *a* being to about half the O_2 content of aerated sea water. 6000 M. C. incandescent source, with $CuSO_4$ filter for infra-red. Large time marks 1 minute apart, small marks on *a*, 1.75 sec.

the solution. Curves of this current vs. applied potential showed a good plateau in the range 0.3 to 0.7 volt, with practically identical values between 0.4 and 0.6 volt, much like those of the dropping cathode. The mid-

value of 0.5 volt was chosen, and the electrode was kept cathodically polarized at this potential. The residual current was then closely proportional to oxygen tension in equilibrium with the solution, between nearly pure N_2 (where practically no current flowed), through air, to 99.5% O_2 . At 0.5 volt applied potential, the O_2 is reduced only to H_2O_2 , the second reduction to H_2O occurring at the second plateau around 1 volt and higher.⁴ But the

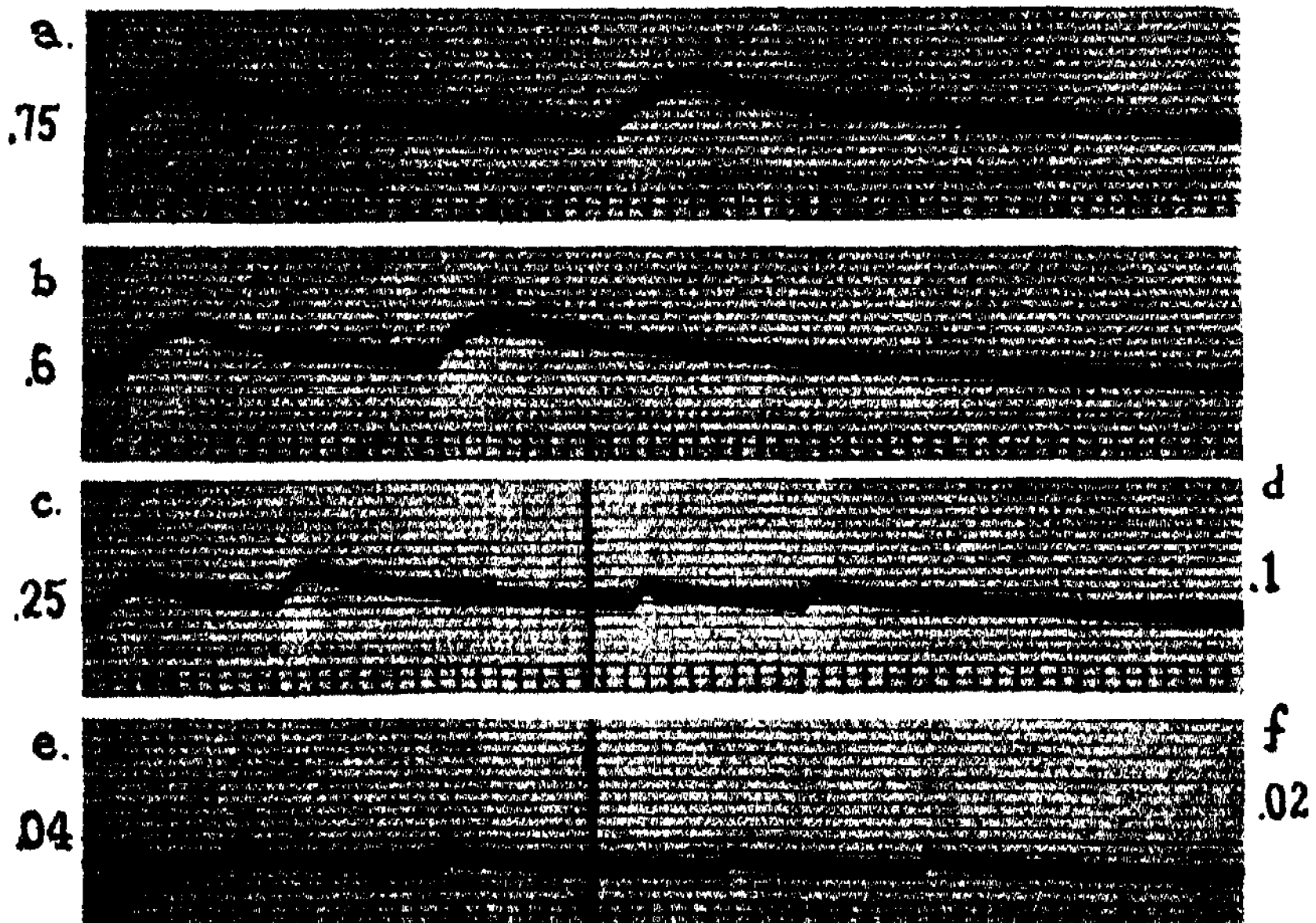


FIGURE 3

Submerged leaf of pondweed (*Potamogeton*), lower surface pressed tightly against a stationary mercury cathode polarized at 0.5 volt. Indicating instrument is a torsion string galvanometer (Kipp), with appropriate amplifier, to show responses to very brief illuminations (6000 M. C., incandescent source, $CuSO_4$ filter for infra-red). Two flashes each of the following durations were given (by camera shutter): *a*: 0.75 sec.; *b*: 0.6 sec.; *c*: 0.25 sec.; *d*: 0.1 sec.; *e*: 0.04 sec.; *f*: 0.02 sec. as indicated on the figure. Decreasing but perceptible responses to each are given, increased O_2 content being upward on the record, as in previous figures. Vertical marks at base of each record are 0.2 sec. apart. Full height of each record represents O_2 content of water in equilibrium with air.

H_2O_2 would be broken down immediately by the catalase of the plant, and it seemed safer to have this happen (as postulated in the normal respiratory process) than run the risk of other substances, such as peroxides themselves, being reduced at the higher potential. Actually the results at 1.0 volt are much like those at 0.5 volt.

In practice, a suspension of cells may be allowed to settle out upon a horizontal electrode, or they may be kept stirred by a vertical electrode in a

closed vessel, if absolute rate measurements are desired. Larger plants (coenocytes, thalli or leaves) may be also stirred in a definite volume, or they may be held tightly against the electrode by an agar block, the back side of the electrode being paraffined to restrict current flow to the contact surface.

After the initial polarization on applying the potential has occurred, the

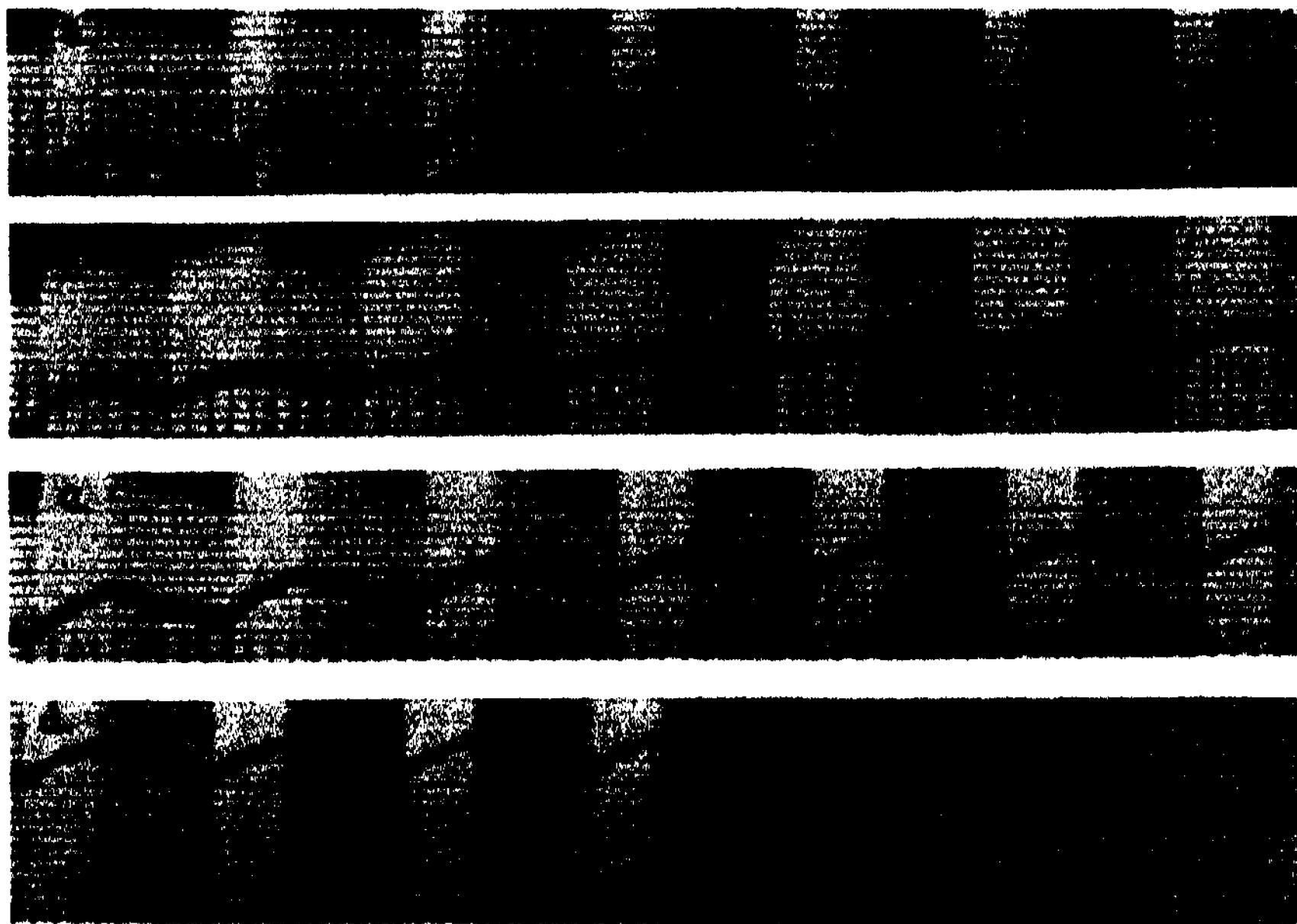


FIGURE 4

Same as figure 3, but with duration of flashes indicated by the paler parts of the record; flashes by revolving disc, interrupting an incandescent source (6000 M. C.) with the following intervals: *a*: 0.45 sec. light, 1.35 sec. dark; *b*: 0.9 sec. light, 0.9 sec. dark; *c*: 0.7 sec. light, 1.1 sec. dark; *d*, later portion of *c*, with long dark period in last half of record. O_2 production begins within the period of the string galvanometer (the deflections of which are rectangular to a calibration voltage at this record speed), and has a nearly constant rate during the flash, or slightly higher at the beginning.

Time marks at base of each record, 0.2 sec. apart. Full height of record *d* represents O_2 content of water in equilibrium with air.

Unretouched print from original negatives, which were illuminated by the same flashes as the plant. First flashes in *a* and *b* slightly shorter than the later ones, as shown by width of pale bands.

current remains steady if the solution is aerated, or drifts slowly downward in a closed vessel as oxygen is consumed by the plant (in the dark) and by the electrode. (The contribution of the latter is determined by a control without the plant.) On illumination there is an increase of current, the response being delayed with the electrode at a slight distance (Fig. 1*b*), but

becoming almost instantaneous when it makes direct contact with the tissue (Fig. 2). Figures 3 and 4, taken with a faster galvanometer, show a practically instant response to light or dark periods. There is no hint of an "induction period," here; indeed, the rate of O_2 evolution appears to be slightly greater during the first moments of illumination than later, although changing diffusion gradients may be responsible for this apparent effect. An induction period is also absent even after rather long dark periods, provided the plant has been kept well aerated. This is in agreement with the findings of other workers,^{7,8} by less rapid methods.

On the other hand, if anaerobic conditions have been reached in the dark (which in leaves might result from stomatal closing at night, and are here quickly attained in the thin film between tissue and electrode by the combined O_2 consumption of both) interesting variations of the time curves are found, as shown in figure 5. After short anaerobic dark periods, there may be only a slight waver or "bayonet angle" in the curve; after longer periods this is seen as a brief cusp preceding the main curve; and after still longer dark periods, many seconds, or even minutes may follow the initial "oxygen gush" before the main trend of O_2 evolution resumes. After an all-night anaerobic dark period the curve may be perfectly flat for many minutes before rising, but it almost invariably shows the initial cusp before flattening.

While the delay in the main curve could be called an "induction period," it seems more reasonable to explain it in the manner suggested by Gaffron,⁷ as due to the accumulation of products of anaerobic respiration or fermentation, which must be oxidized by the liberated O_2 before the latter becomes manifested as such (whether by pressure or electrical methods). The initial cusp may therefore show that such oxidation is slightly slower in starting than is the evolution of oxygen.

Some uncertainty must be admitted as to the nature of this initial cusp. We have no sure means of proving that it is oxygen, instead of some other substance reducible at the electrode at this low potential. But there is no evidence of such a substance either on the polarographic or photosynthetic side, and it is not likely that a substance would be released from the cells and diffuse to the electrode so rapidly unless it were a gas. Since Gaffron's curves,⁷ taken with a gas pressure method, show a broader though unmistakable cusp of the same sort, we prefer to take the simplest view, that the electrode cusp is also due to oxygen.

However, the cusp might be oxygen evolved by some light reaction not strictly photosynthetic. Heating seems to be ruled out, since infra-red alone causes a slow drift in the opposite direction; and the reaction is not shown by non-photosynthetic organisms, such as yeast or albino leaves. Possible complications due to guard cells and air spaces in leaves no doubt exist, but since marine algae also show the oxygen gush very well, it seems to be a general phenomenon in green plants.

It is tempting to compare the oxygen gush with the "acid gush" found with the glass electrode.¹ They occur under the same conditions, after a long dark period, and especially anaerobically, and coincide well in duration. They may be wholly independent, but it is possible to postulate a connection. Thus the oxygen gush, acting upon anaerobically accumulated substances permitting an R. Q. greater than 1.0, would release more CO₂ than was being simultaneously assimilated photosynthetically.⁷ Con-

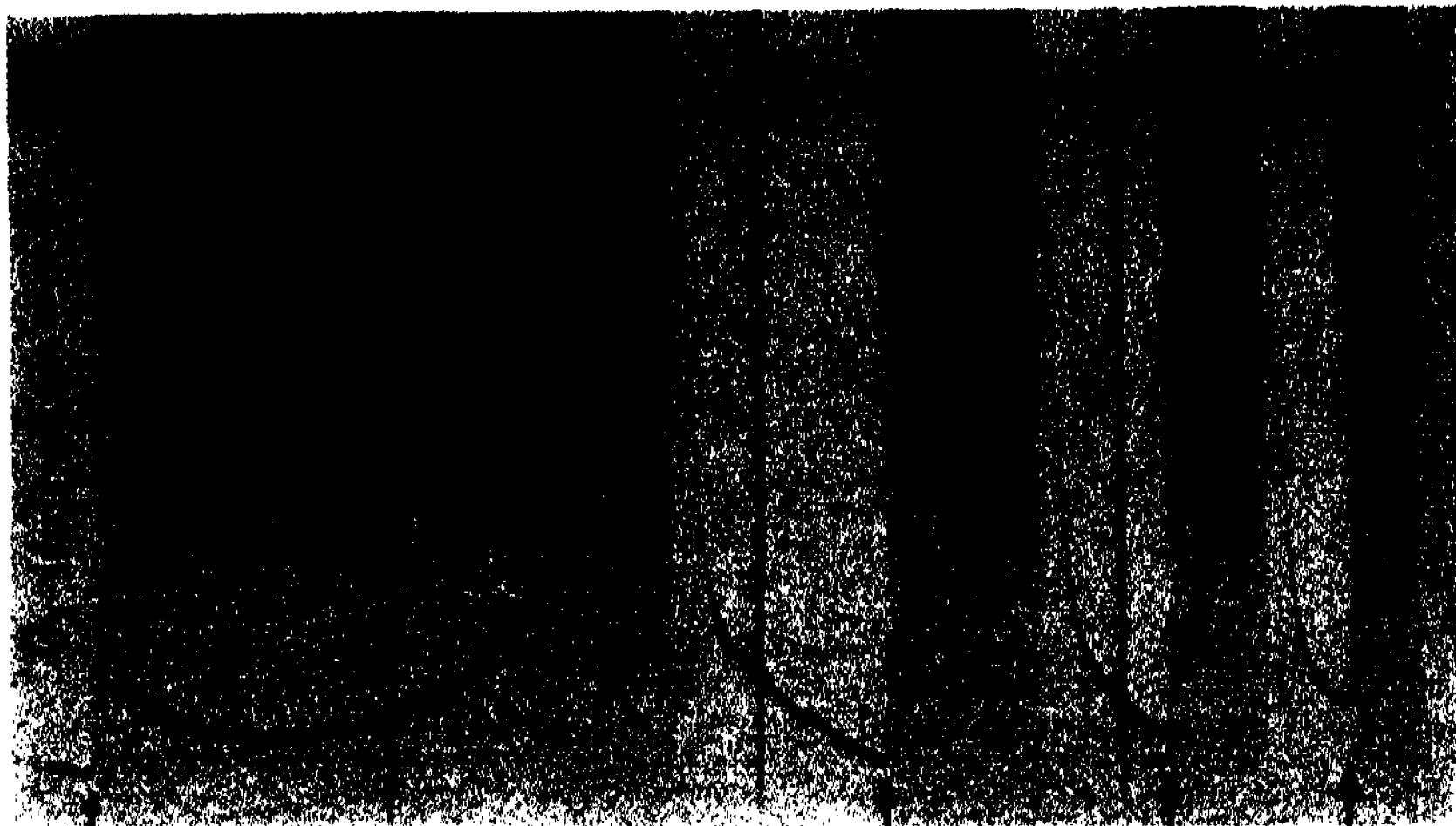


FIGURE 5

Castor bean (*Ricinus*) leaf, lower surface pressed tightly (by thin agar plug) against a stationary mercury cathode, polarized at 0.5 volt. Current through series galvanometer proportional to O₂ content of water film, increased O₂ being upward on the record. The O₂ content had drifted down to nearly anaerobic levels through the combined effect of respiration, and of reduction by the electrode. The leaf was then illuminated (through the agar) with incandescent light of about 7500 M. C. during the shaded parts of the record: in *a* after a 10 minute dark period, in *b* after 34 seconds, in *c* after 21 seconds and in *d* after 13.5 seconds dark periods, respectively. Note the immediate gush of O₂ production in each case, followed by a delay after long dark periods, but becoming a mere inflection in the rising curve after short dark periods.

Heavy vertical lines are time marks, one minute apart. Arrows indicate beginning of illumination. Essentially similar records were obtained with a platinum electrode, and with marine algae (e.g., *Ulva*) without stomata or gas spaces.

versely the acid gush might release CO₂ in large quantity, directly or from carbonates, allowing photosynthesis and O₂ evolution to start at a higher rate. Gaffron's work suggests that the former is the more likely situation.

On the other hand the oxygen records show no anomaly on darkening after long illumination, to correspond with the "alkaline gush" found with the glass electrode (which temporarily carries the pH change in a direction opposite to its later drift). On darkening, the O₂ content of the water film in-

stantly drops, at first with a greatly enhanced rate, which may indicate an increased respiration (although altered diffusion gradients must be allowed for). This may be a valuable correction to apply in determining the true photosynthetic rate for quantum efficiency and other determinations. Other correlations, as with flashing light experiments, fluorescence decay, etc., are obvious. The connection with certain bio-electric effects, which were the original impetus in developing the method, will be discussed in another paper.

¹ Blinks, L. R., and Skow, R. K., *Proc. Nat. Acad. Sci.*, **24**, 413-419 (1938).

² See Spoehr, H. A., *Photosynthesis*, New York, 228-229 (1926).

³ Tang, P. S., and Lin, C. Y., *Jour. Cell. and Comp. Physiol.*, **9**, 149 (1936), employed a platinum electrode in a suspension of *Chlorella* cells to follow the changes of oxidation-reduction potential during photosynthesis, but air was constantly bubbled through the suspension, so that changes in oxygen concentration probably did not occur. The observed change was of some other sort, upon an unknown oxidation-reduction system, probably cellular metabolites.

⁴ Vitek, V., *Collection Czechoslovak Chem. Commun.*, **7**, 537 (1935); see also *Chimie et Industrie*, **29**, 215 (1933).

⁵ Baumberger, J. P., and Müller, O., reported at winter meetings of Western Society of Naturalists, Stanford University, 1935, and at IX Int. Physiol. Congress, Zürich (1938).

⁶ Petering, H. G., and Daniels, F. (in press) and reported at meetings of the Electrochemical Society, Dallas (1938), and American Chemical Society, Milwaukee (1938).

⁷ Gaffron, H., *Biochem. Zeit.*, **280**, 337 (1935); *Naturwissenschaften*, **25**, 460, 715 (1937). Also unpublished experiments and suggestions.

⁸ Emerson, R., *Ann. Rev. Biochem.*, **6**, 539 (1937).

REVERSAL OF THE POTASSIUM EFFECT IN NITELLA

By W. J. V. OSTERHOUT AND S. E. HILL

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated August 17, 1938

The striking change of P.D. in a negative direction produced by potassium in *Nitella* has been called for convenience the potassium effect. It disappears¹ when cells are leached in distilled water. In such cells the effect is sometimes reversed so that replacement of 0.01 M NaCl by 0.01 M KCl makes the P.D. more positive, as shown in figure 1.²

This was shown in a previous paper³ but because it was infrequent it was passed over without comment. Since then cases have multiplied so that there is no doubt that it occurs regularly under certain conditions.

Regarding these conditions not much can be said but the reversal appears to be favored by prolonged leaching.

In such cells the concentration effects of KCl and NaCl are practically alike, both being about 22 mv. with dilute solution positive in the external circuit.⁴ We therefore suppose that the apparent mobilities of K^+ and Na^+ are approximately equal and attribute⁵ the fact that KCl is positive to NaCl to a higher partition coefficient, S , for NaCl than for KCl ($S = \text{concentration in } X, \text{ the outer non-aqueous protoplasmic surface layer} \div \text{concentration in the external medium (cf. figure 2)}$)).

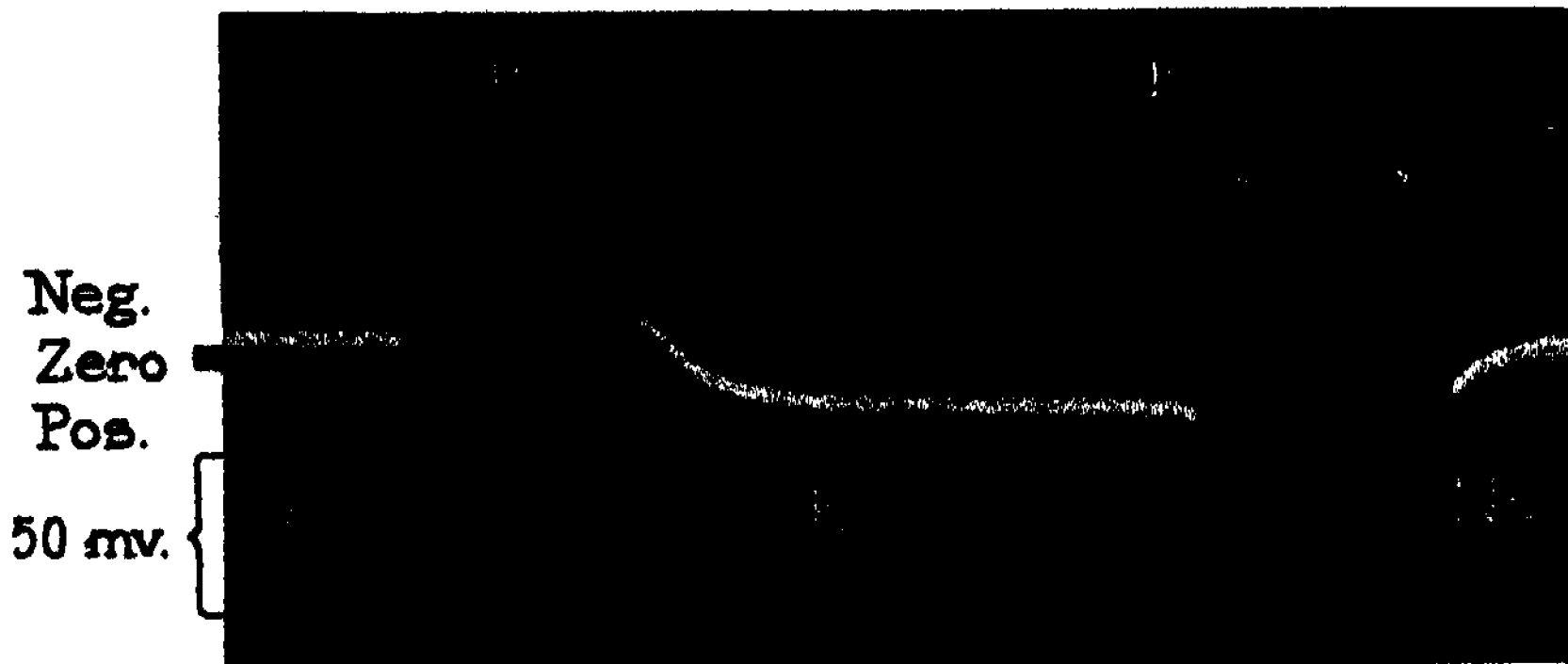


FIGURE 1

Photographic record showing that with a leached cell the substitution of KCl for NaCl may make the P.D. more positive (contrary to the effect on unleached cells).

The leads were arranged as in figure 2: C is recorded (F was in contact with 0.01 M NaCl).

At the start C was in contact with 0.01 M NaCl: this solution was removed and the curve jumped to F , the "free grid" of the vacuum tube amplifier. When 0.01 M KCl was placed in contact with C the curve jumped back and then began to fall indicating that the P.D. was becoming more positive: this continued until it was 25 mv. more positive.

This solution was removed and the curve jumped back to F but when 0.01 M NaCl was applied the curve jumped back and began to rise, returning nearly to the original value.

The cell was freed from neighboring cells and kept for some days in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *Jour. Gen. Physiol.*, 17, 87 (1933-34)) at $15 \pm 1^\circ$. It was then kept for 3 days in distilled water at the same temperature. The measurements were made at $22^\circ C$.

Time scale 1.8 mm. per second.

In normal unleached cells the partition coefficients may be equal⁴ or that of KCl may be greater than that of NaCl.⁶ Such differences in partition coefficients do not seem surprising in view of certain experiments with models which show that KCl is positive to NaCl with some non-aqueous liquids and negative with others. It would therefore seem that the behavior of the outer non-aqueous surface layer of the protoplasm might show either situation depending on the metabolism of the cell.

Taking the average as 22 mv. for the change produced by substituting 0.01 *M* for 0.001 *M* KCl we may write (for 20°C.)

$$22 = 58 \frac{u - v}{u + v} \log \frac{C'}{C''}$$

where C' and C'' are concentrations and u and v are the mobilities of the cation and anion, respectively. All values relate to X , the outer non-aqueous protoplasmic surface layer (Fig. 2). Putting $C' \div C'' = 10$ and $v = 1$ we obtain $u = 2.22$. Since the concentration effect of NaCl is approximately the same as that of KCl we may put $u_K = u_{Na} = 2.22$.

The effect of substituting 0.01 *M* KCl for 0.01 *M* NaCl may be calculated by means of Henderson's equation which may be written (for 20°C.)

$$\text{P.D.} = 58 \frac{(U_I - V_I) - (U_{II} - V_{II})}{(U_I + V_I) - (U_{II} + V_{II})} \log \frac{U_I + V_I}{U_{II} + V_{II}}$$

where $U_I = C_K u_K$, $V_I = C_{KCl} v_{Cl}$, $U_{II} = C_{Na} u_{Na}$ and $V_{II} = C_{NaCl} v_{Cl}$. In the present case where $u_{Na} = u_K = u$ this reduces⁶ to

$$\text{P.D.} = 58 \frac{u - v}{u + v} \log \left(\frac{C_{NaCl}}{C_{KCl}} = \frac{S_{NaCl}}{S_{KCl}} \right).$$

If we put for the ratio of partition coefficients $S_{NaCl} \div S_{KCl} = 8$ this gives for the P.D. 20 mv. with KCl positive to NaCl, which agrees with observation.

If we make such calculations for different values of $u_{Na} = u_K$ with $S_{NaCl} \div S_{KCl} = 8$ we obtain the curve shown in figure 3 (other curves are added for comparison).⁷

In normal unleached cells of this same lot we have found⁶ the values $u_K = 11.9$, $u_{Na} = 7.93$, $S_{KCl} \div S_{NaCl} = 60$. It thus appears that leach-

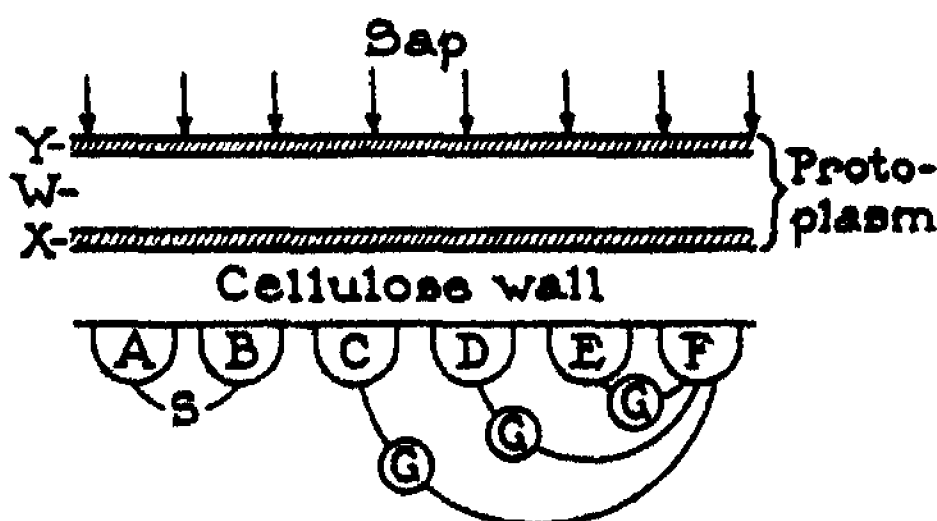


FIGURE 2

Diagram to show the arrangement of leads and the supposed structure of the protoplasm which is assumed to consist of an aqueous layer W , an outer non-aqueous layer X and an inner non-aqueous layer Y .

The arrows show the outwardly directed (positive) P.D. whose seat is supposed to be chiefly at Y when the cell is in pond water; hence the P.D. at X is regarded as negligible and is not shown. But under some conditions the P.D. at X may become important.

Each lead is connected to a separate amplifier and to one string of the 3-string Einthoven galvanometer.

ing with distilled water may affect both the mobilities and the partition coefficients. It is to be hoped that further studies may enable us to find other methods of controlling these important variables. (In this connection we may recall that guaiacol lowers the mobility of K^+ in *Valonia*⁸ and increases the mobility of Na^+ in *Valonia* and in *Halicystis*,⁹ and calcium lowers the partition coefficient of KCl in *Nitella*.¹⁰)

Summary.—In normal cells of *Nitella* KCl is strongly negative to NaCl (potassium effect). But in certain cells leached in distilled water this effect is reversed and KCl becomes positive to NaCl.

Since the concentration effect indicates that u_{Na} and u_K (the mobilities

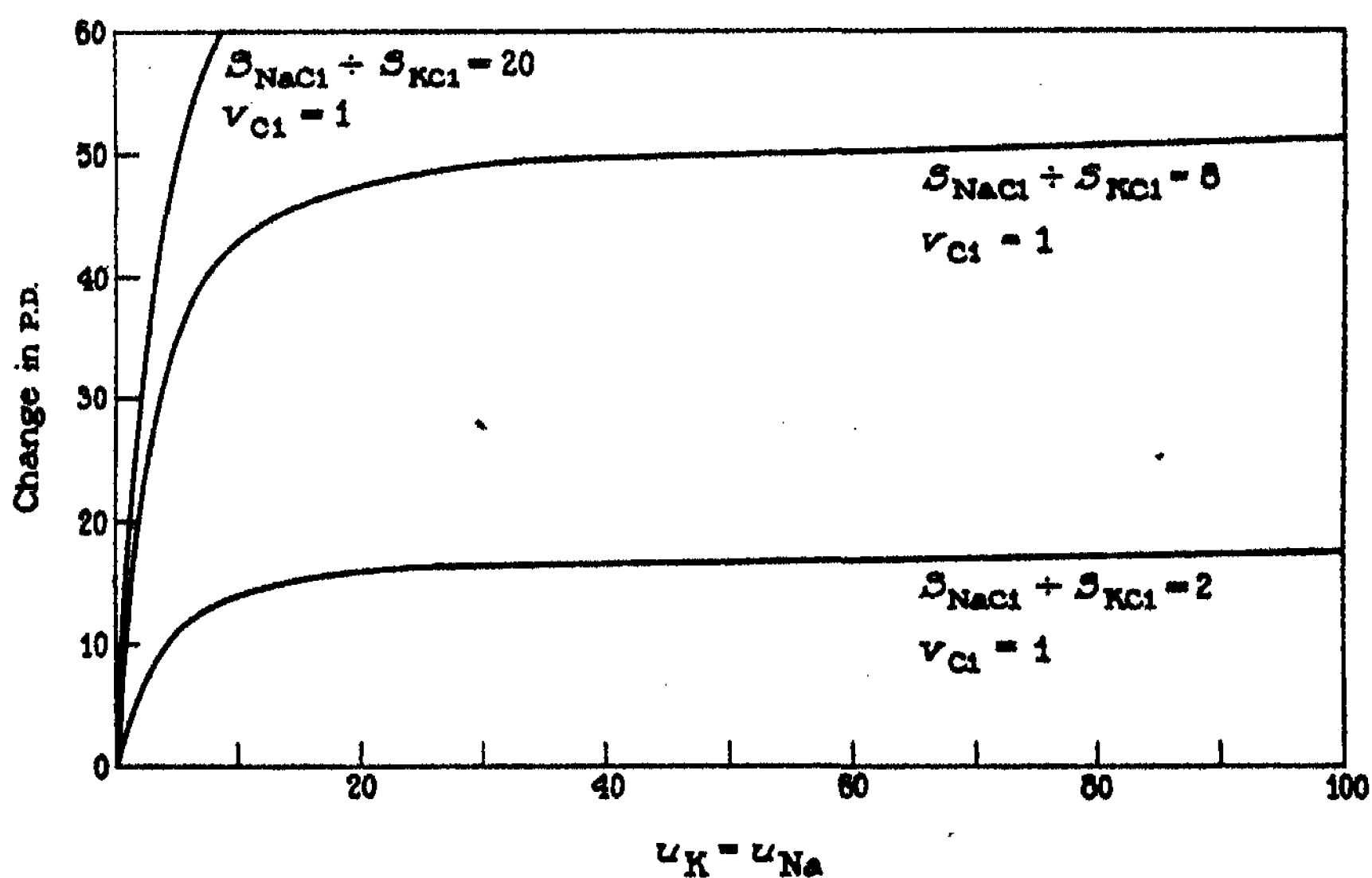


FIGURE 3

Shows the calculated values of the change in P.D. produced by substituting 0.01 *M* KCl for 0.01 *M* NaCl when u_{Na} and u_K (the mobilities of Na^+ and K^+ in the outer non-aqueous protoplasmic surface layer) are equal and the ratios of the partition coefficients, $S_{NaCl} + S_{KCl}$, are 2, 8 and 20. The mobility of Cl^- (v_{Cl}) is taken as unity.

of Na^+ and K^+ in the outer non-aqueous protoplasmic surface layer) are approximately equal, the reversal may be attributed to a higher partition coefficient for NaCl than for KCl. In unleached cells K^+ has a higher mobility than Na^+ and its partition coefficient, S , is equal to or greater than that of Na^+ ($S = \text{concentration in the outer non-aqueous protoplasmic surface layer} \div \text{concentration in the external medium}$).

The experiments indicate that both the mobility and the partition coefficient can be changed by treatment with distilled water.

¹ Osterhout, W. J. V., and Hill, S. E., *Jour. Gen. Physiol.*, 17, 105 (1933-34).

² The experiments were made on *Nitella flexilis*, Ag., using the technique described

in former papers (cf. Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 967 (1933); Hill, S. E., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, **21**, 541 (1937-38); Blinks, L. R., *Ibid.*, **13**, 495 (1929-30).

³ Osterhout, W. J. V., *Ibid.*, **18**, 987, figure 2 (1934-35).

⁴ Osterhout, W. J. V., *Ibid.*, **13**, 715 (1929-30).

⁵ Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sci.*, **24**, 312 (1938).

⁶ We may write

$$\begin{aligned} \text{P.D.} &= 58 \frac{(uC_{Na} - vC_{Na}) - (uC_K - vC_K)}{(uC_{Na} + vC_{Na}) - (uC_K + vC_K)} \log \frac{uC_{Na} + vC_{Na}}{uC_K + vC_K} \\ &= 58 \frac{(u - v)(C_{Na} - C_K)}{(u + v)(C_{Na} + C_K)} \log \frac{C_{Na}(u + v)}{C_K(u + v)} \\ &= 58 \frac{u - v}{u + v} \log \frac{C_{Na}}{C_K} \end{aligned}$$

⁷ As $u_{Na} = u_K = u$ increases the P.D. approaches the limiting value $\text{P.D.} = 58 \log \frac{C_{Na}}{C_K}$.

⁸ Osterhout, W. J. V., *Jour. Gen. Physiol.*, **20**, 13 (1936-37).

⁹ Osterhout, W. J. V., *Ibid.*, **21**, 707 (1937-38).

¹⁰ Unpublished.

SYNTHESES CARRIED OUT IN VIVO BY ISOLATED PEA ROOTS: I

BY JAMES BONNER AND EDWIN R. BUCHMAN

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES AND GATES AND
CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated September 12, 1938

There has long been interest in the mechanisms by which chemical reactions take place *in vivo*, and numerous techniques have been applied to the study of this problem. A precise interpretation of purely analytical results is, however, often difficult or impossible, particularly if the reactions in question have been allowed to take place in the intact organism. It is desired to present in the present paper a few of the results obtained with the aid of a new experimental approach to the problem of physiological syntheses. A single relatively simple organ has been cultivated *in vitro* under conditions which have been closely controlled both as to external environment and as to nutrient supply, and the metabolism related to a single well defined and readily determinable substance has been investigated.

In earlier papers^{1,2} it has been shown that isolated pea roots may be successfully grown *in vitro* provided only that a suitable nutrient medium is used. Such a nutrient medium must of course contain carbohydrates (in

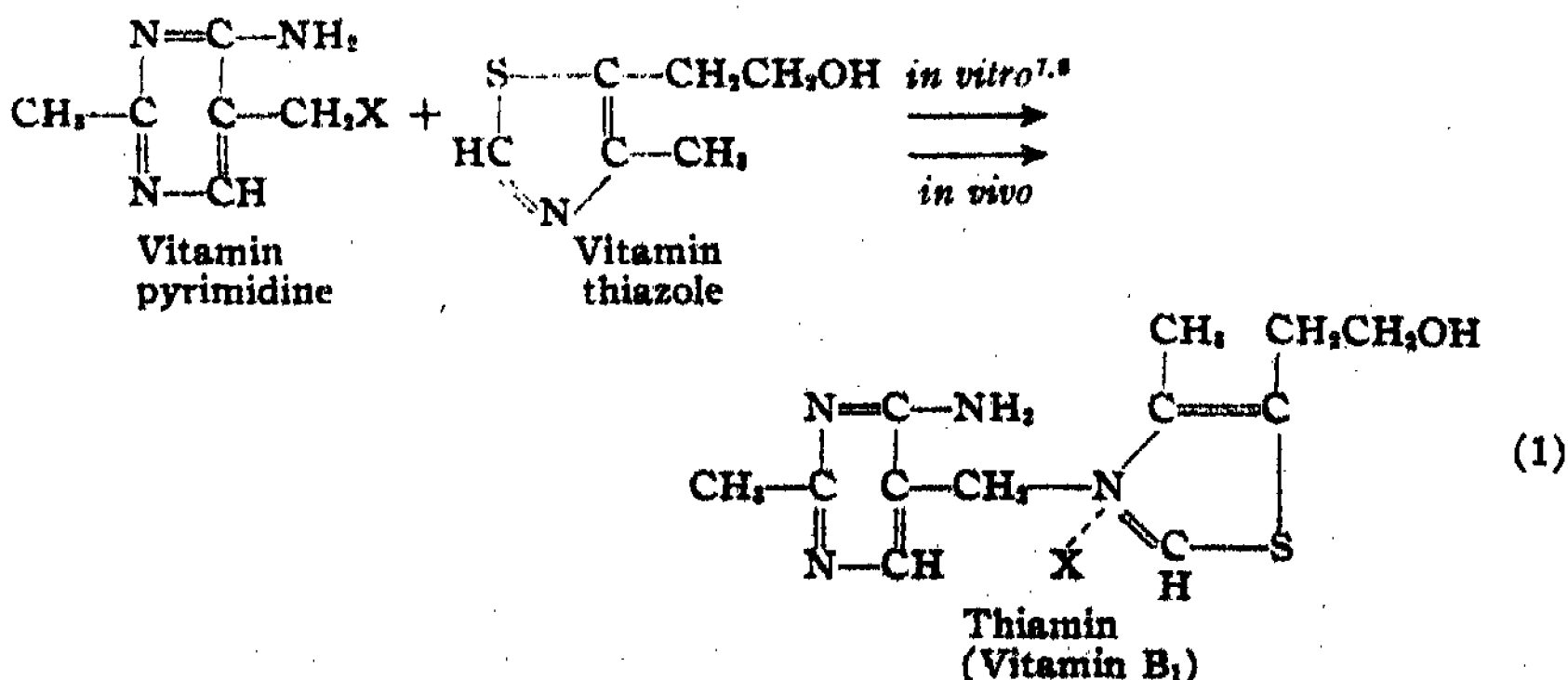
this case sucrose), a source of nitrogen (in this case nitrate) and the correct kinds and proportions of inorganic salts. The medium must in addition contain a small amount, 0.1 mg. per liter or less, of vitamin B₁ if continued growth of the root is to occur. It is with the metabolism of vitamin B₁ and related substances that the present paper is concerned.

It has been found possible³ to replace B₁ by various other similarly constituted substances or combinations of such materials. The question naturally arises whether or not these substances are transformed by the organism into the vitamin itself. An answer can be obtained if the rate of formation of B₁ *in vivo* is substantially greater than the rate of its destruction and if an assay method can be made available which is specific for the vitamin molecule. Both of these conditions are well enough met in the cases to be discussed so that some picture can be presented of modes and limitations of such syntheses as effected by isolated pea roots.

The methods used have been described in detail elsewhere^{2,3,4} and need not be gone into here. Three general types of measurements have been made:

1. Ability of substances related to vitamin B₁ to replace the latter in supporting the growth of excised pea roots.
2. A quantitative assay with the fungus *Phytophthora cinnamomi*, which is used for the determination of amounts of vitamin B₁.⁵
3. A quantitative assay with the fungus *Phycomyces Blakesleeanus*, which is used for the determination of the total of vitamin B₁ and/or free vitamin pyrimidine plus vitamin thiazole.^{4,6}

The vitamin B₁ molecule contains a substituted pyrimidine nucleus linked through a methylene bridge to a substituted thiazole nucleus. These two portions, which will be referred to hereafter as the "vitamin pyrimidine" and the "vitamin thiazole," may be linked *in vitro* to form the vitamin itself. In order that this condensation (Reaction (1)) take place in the test tube it is essential that the 5-methyl group of the pyrimidine be substituted with a reactive group X, such as a Br atom.



It has been shown⁹ that numerous organisms which require vitamin B₁ as an accessory growth factor are able to utilize a mixture of the vitamin pyrimidine and the vitamin thiazole in place of the vitamin itself. In fact, pyrimidines which cannot *in vitro* be linked with the vitamin thiazole, such as the 5-aminomethyl pyrimidine ($X = NH_2$), may even be used as the pyrimidine component for the growth of certain microorganisms and for the pea root.⁸ It has been generally supposed that these organisms actually combine the pyrimidine and thiazole halves *in vivo* to form the vitamin. In no case, however, has such a synthesis been actually demonstrated. Evidence that the pea root does in fact synthesize vitamin B₁ was obtained from experiments in which pea roots were supplied with a mixture of the 5-aminomethyl pyrimidine and the vitamin thiazole. After the roots had grown with this mixture as their growth factor, root tips were removed and assayed (a) by the *Phytophthora* test, which determines the vitamin B₁,⁶ but not the pyrimidine-thiazole mixture, and (b) by the *Phycomyces* test, which determines vitamin B₁ and/or any of the uncombined intermediates. Table 1 shows that the roots contain only vitamin B₁ and no significant amount of the uncombined intermediates.¹⁰

TABLE 1
VITAMIN B₁ CONTENT OF PEA ROOT TIPS AFTER CULTIVATION WITH VARIOUS GROWTH FACTORS

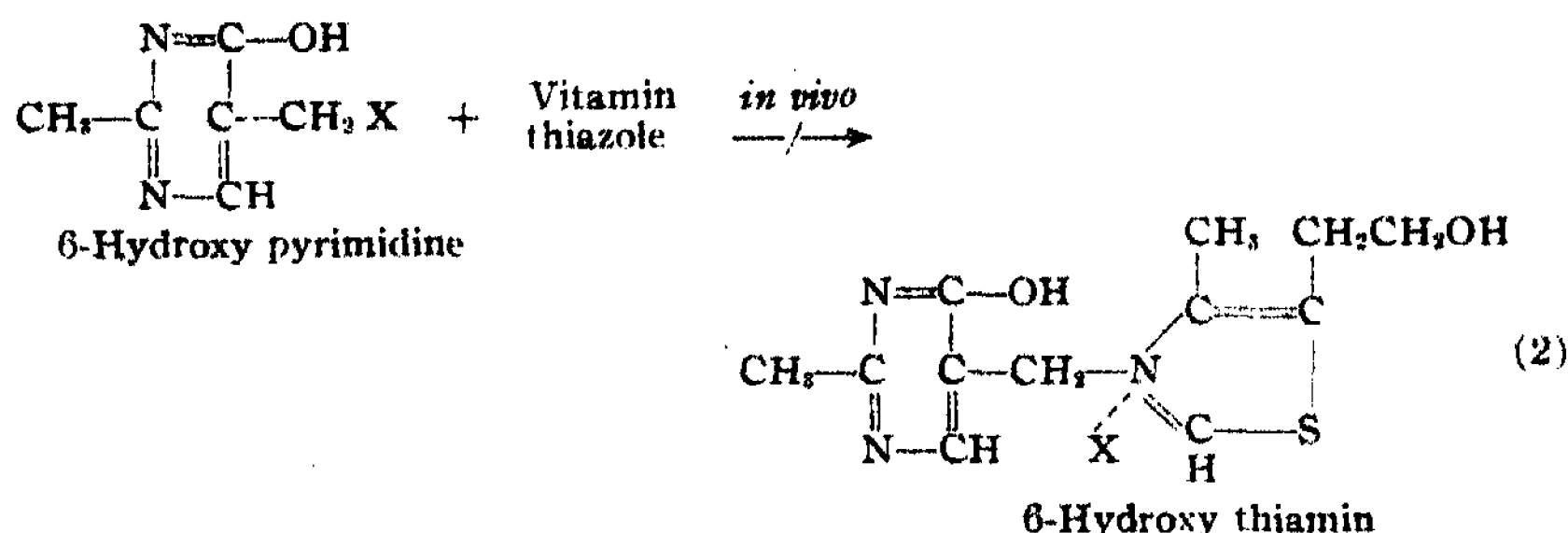
CONTENT OF	GROWTH FACTOR SUPPLIED		
	NONE	PYRIMIDINE + THIAZOLE	VITAMIN B ₁
Vitamin B ₁ (<i>Phytophthora</i> assay)	0	100%	100%
Vitamin B ₁ and/or pyrimidine + thiazole (<i>Phycomyces</i> assay)	0	100%	100%
Pyrimidine + thiazole (difference of the two assays)	0	insignificant	insignificant

It is also of interest that the roots supplied with the mixture of intermediates contain as much of the vitamin as do roots supplied with the vitamin itself. Control roots contain no appreciable amount of the vitamin. This means, then, on the assumption made in footnote 5, that the pea root must be capable of conducting *in vivo* a synthesis of the vitamin which does not take place in the test tube and we postulate that this synthesis is due to the action of a specific enzyme¹¹ furnished by the organism.

It was further found, by experiments similar to these, that the root is also able to synthesize vitamin B₁ in the presence of the vitamin thiazole, from pyrimidines in which the 5-methyl group is substituted with either an ethoxy ($X = OC_2H_5$) or a thioformamido group ($X = NHCSH$).

By means of what we shall term "competition" experiments, it was found possible to study the limitations of this *in vivo* coupling of pyrimidine and

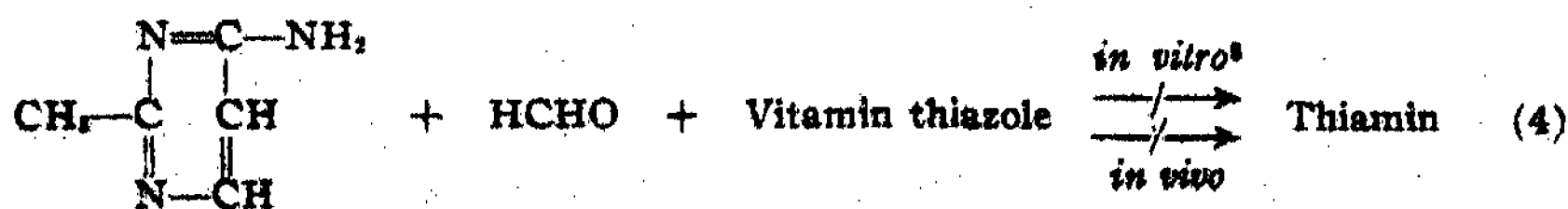
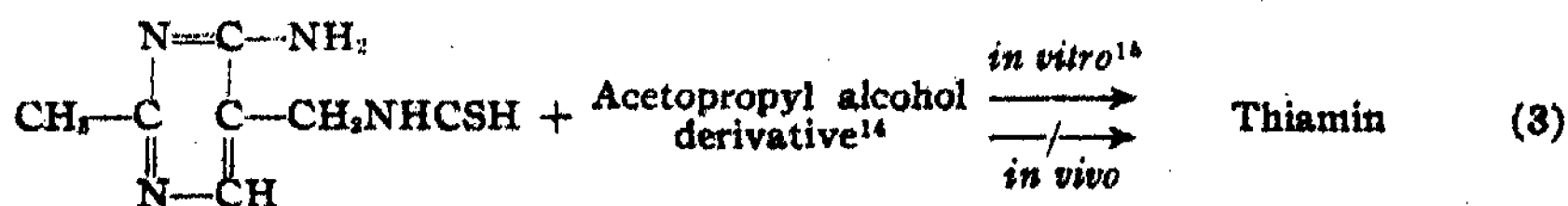
thiazole by roots. In this paper the application of this tool to the study of the following reaction is pointed out; it should be noted, however, that the method is capable of considerably wider application.



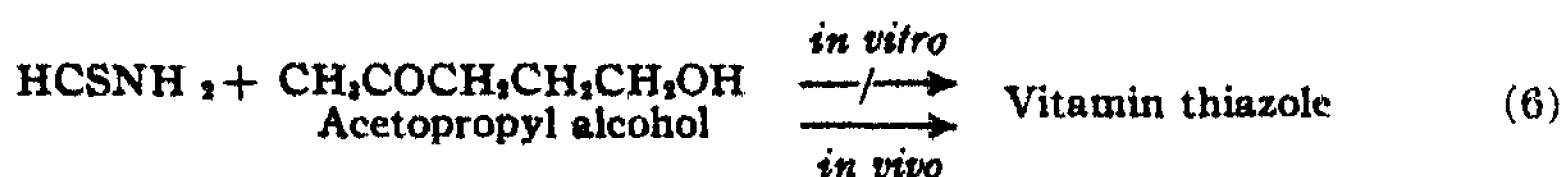
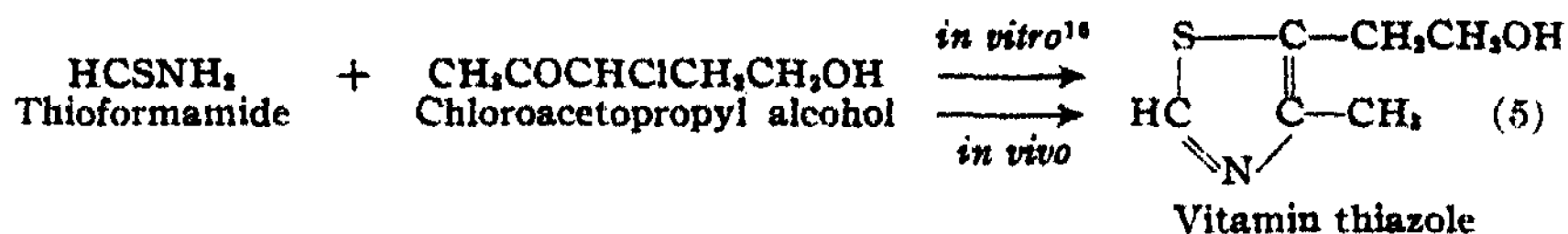
$\xrightarrow{\text{in vivo}}$ indicates that the reaction does not take place under the conditions specified.

6-Hydroxy vitamin does not support the growth of pea roots¹² either alone or in combination with vitamin pyrimidine.¹³ Therefore this vitamin analog does not break up appreciably *in vivo* to make the vitamin thiazole available for recombination. Moreover, when 6-OH pyrimidine ($\text{X} = \text{OC}_2\text{H}_5$) is added, even in large excess, to a mixture of vitamin pyrimidine ($\text{X} = \text{OC}_2\text{H}_5$) and vitamin thiazole, it is found to exert no effect on the activity of the mixture in supporting the growth of pea roots. Reasoning from these results, the stability of the inactive vitamin analog and the inability of the inactive pyrimidine to "compete" with the active pyrimidine for the available vitamin thiazole, we conclude that an enzymatic synthesis (Reaction (2)) of the 6-OH vitamin is not accomplished at a detectable rate *in vivo*. Hence the enzyme system which we assume to be responsible for *in vivo* synthesis of the vitamin exhibits a certain degree of specificity in its action. Numerous analogs of the vitamin thiazole are active as the thiazole component in supporting the growth of pea roots and hence are presumably synthesized to vitamin analogs *in vivo*, so that the specificity of the above enzyme system is by no means complete.

The fact that it has not been found possible to obtain evidence for an *in vivo* production of thiamin according to reactions (3) and (4) lends further weight to the view that the vitamin is actually produced in nature by a simple joining of its two halves.



The pea root is unable to synthesize either vitamin pyrimidine or vitamin thiazole from the sucrose and the inorganic constituents of the basic nutrient medium. We have found that it is, however, able to synthesize the vitamin thiazole from appropriate simpler substances. Of the two reactions given here for formation of the thiazole only the first one takes place in the test tube whereas pea roots can be shown to accomplish both of them.



When supplied with a mixture of vitamin pyrimidine, thioformamide,¹⁷ and either chloroacetopropyl alcohol or acetopropyl alcohol, the roots grow as well and contain as much thiamin (as judged by the *Phycomyces* assay) as roots supplied with vitamin B₁ itself.

TABLE 2
VITAMIN B₁ CONTENT OF PEA ROOT TIPS AFTER CULTIVATION WITH VITAMIN PYRIMIDINE
AND ACYCLIC THIAZOLE INTERMEDIATES

	GROWTH FACTOR SUPPLIED			
	NONE	PYRIMIDINE + VITAMIN THIAZOLE	PYRIMIDINE + THIOFORMAMIDE + CHLOROACETO- PROPYL ALCOHOL (REACTION (5))	PYRIMIDINE + THIOFORMAMIDE + ACETOPROPYL ALCOHOL (REACTION (6))
Vitamin B ₁ content (<i>Phycomyces</i> assay)	0	100%	100%	100%

Control experiments with *Phycomyces* show that this organism cannot in either case utilize the thiazole intermediates to replace the vitamin thiazole under the present conditions. This, then, justifies the use of the *Phycomyces* assay in the experiments of table 2 and also indicates that under our conditions the synthesis of thiazole according to Reaction (5) is enzymatic in nature. It may be concluded that the pea root is able to effect a ring closure from thioformamide and either acetopropyl alcohol or its chloro derivative with the formation of the vitamin thiazole. It would seem not unlikely that the synthesis of the thiazole ring in nature is accomplished in a similar manner.

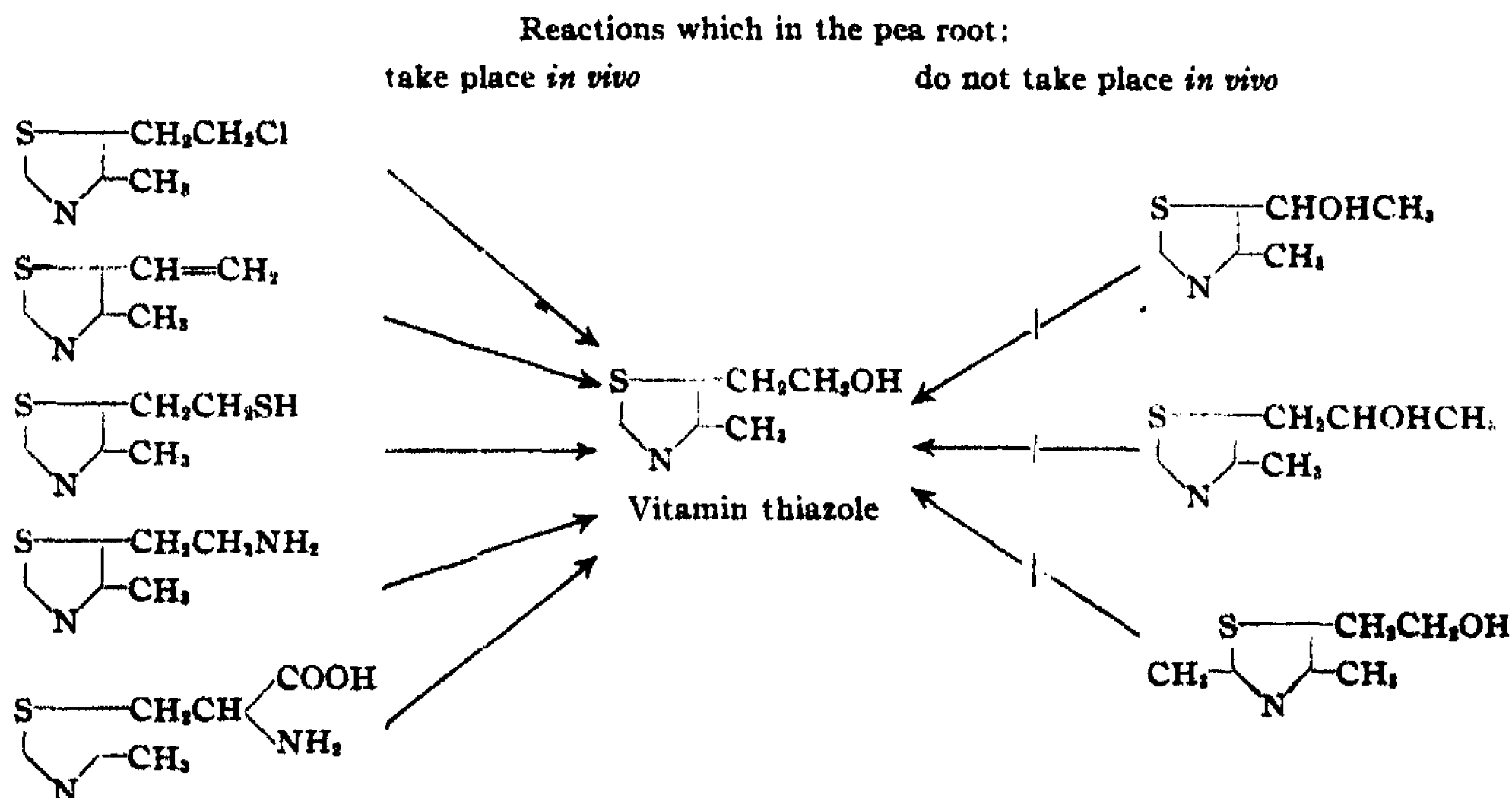
The 4-methyl, 5- α -hydroxyethyl thiazole possesses a considerable activity as the thiazole component for the growth of pea roots. The synthesis

of this analog of the vitamin thiazole can be formulated as follows:



Such a synthesis is not, however, effected by pea roots, i.e., although the roots are apparently able to utilize this analog if it is supplied to them ready made, they are unable to synthesize it as they are the vitamin thiazole. This indicates that also in the case of the enzyme system responsible for thiazole synthesis there is a considerable specificity.

A number of analogs of the vitamin thiazole are highly active as the thiazole component for the growth of isolated pea roots. In the figure are



shown, at the left, five such analogs¹⁸ each of which could conceivably be converted *in vivo* by the organism to the vitamin thiazole, shown in the center. Such a transformation actually does take place; roots supplied with vitamin pyrimidine plus any one of these five analogs of the vitamin thiazole not only grow as well, but also contain essentially the same amount of vitamin B₁ as do roots grown upon the vitamin itself. The vitamin assays were carried out in these cases by the *Phycomyces* method which is permissible¹⁹ since the five analogs under discussion have themselves activities upon the growth of *Phycomyces* which are much smaller than that of the vitamin thiazole.⁴

The three thiazole analogs¹⁸ which are shown at the right of the figure are also active in supporting the growth of pea roots. It would seem *a priori* unlikely from a chemical standpoint that any of these should be

metabolized to the vitamin thiazole by the root. Experiment shows that in no case do pea roots which have *grown* with one of these analogs as the thiazole component of the medium contain any significant amount of substance active in supporting the growth of *Phycomyces*. It must therefore be concluded that indeed no *in vivo* conversion to the vitamin thiazole takes place in these cases and consequently that each of these three thiazoles can form a part of a hormone molecule differing in structure from, but having physiological properties similar to the natural vitamin.

It has been shown in the present paper that the pea root synthesizes vitamin B₁ (or a substance indistinguishable from vitamin B₁ by the *Phytophthora* bioassay) from a mixture of the pyrimidine and thiazole components of the vitamin molecule, and that this reaction is carried out *in vivo* under conditions such that no *in vitro* reaction can occur. There is a considerable specificity as to the structures of the pyrimidine and thiazole which may take part. This must then be a synthesis in which a specific enzyme, a "thiaminase" (from "thiamin," the chemical name proposed for the vitamin²⁰), takes part. A second and distinct enzyme system is able to effect closure of the thiazole ring from suitable acyclic substances to form the vitamin thiazole. Since analogs of the vitamin thiazole are apparently not formed in analogous fashion, this "thiazolase" must also be somewhat specific in its action. The suggestion is made that both "thiaminase" and "thiazolase" play a part in the natural synthesis of thiamin by the plant. It has further been shown that certain thiazole derivatives are transformed to the vitamin thiazole *in vivo* by enzymatic reactions corresponding to deamination, decarboxylation, hydrolysis and hydration; whereas certain other growth-promoting thiazoles are not so transformed. It is suggested that the methods outlined in the present paper may offer a new and more exact approach to the problem of the mechanism of biosyntheses.

Acknowledgment.—The chemical portion of this work has been made possible through a grant from the Research Corporation, for which the authors express their gratitude. The biological testing was carried out with the aid of the Works Progress Administration, Official Project number 465-03-3-342, Work Project N-9199.

¹ Bonner, J., *Science*, **85**, 183 (1937).

² Bonner, J., and Addicott, F., *Bot. Gaz.*, **99**, 144 (1937).

³ Bonner, J., *Amer. Jour. Bot.*, **25**, 543 (1938).

⁴ Bonner, J., and Erickson, J., *Amer. Jour. Bot.* (in press).

⁵ Robbins, W. J., *Proc. Nat. Acad. Sci.*, **24**, 53 (1938). Experiments in this laboratory have confirmed this report that *Phytophthora* responds to vitamin B₁ but not to a mixture of vitamin pyrimidine plus vitamin thiazole. The *Phytophthora* assay will be assumed here, then, to determine vitamin B₁ and not the mixture of intermediates. It may be that this assay determines combined forms of the vitamin, such as co-carboxylase, as well, but this, if true, would not alter the arguments presented here.

⁶ Schopfer, W. H., and Jung, A., *Compt. rend. 5ème Congrès Intern. tech. et chim. Ind. agr., Scheveningue, 1937*, 22.

⁷ Williams, R. R., and Cline, J. K., *Jour. Amer. Chem. Soc.*, **58**, 1504 (1936)

⁸ Cline, J. K., Williams, R. R., and Finkelstein, J., *Jour. Amer. Chem. Soc.*, **59**, 1052 (1937).

⁹ First demonstrated for *Staphylococcus aureus*: Knight, B. C. J. G., *Biochem. Jour.*, **31**, 986 (1937); see reference 3, and review by Schopfer, W. H., *Arch. Mikrobiol.*, **9**, 116 (1938).

¹⁰ The average amount of vitamin B₁ found in one root tip 1 cm. long, cultivated for one week in nutrient medium containing either vitamin B₁ or a vitamin pyrimidine-vitamin thiazole mixture, was $6 \times 10^{-4} \pm 0.65 \times 10^{-4}$ mgs. In tables 1 and 2, relative values are used for the sake of simplicity. "100%" indicates no experimentally significant difference from the vitamin B₁ control tips. "0%" indicates no experimentally significant amount of vitamin B₁ or its intermediates.

¹¹ Robbins, W. J. (see reference 5) has suggested "that the synthesis of the vitamin from its intermediates is enzymatic."

¹² The inability of 6-hydroxy vitamin and other vitamin analogs (see reference 3) to support the growth of pea roots is evidence that the organism is *unable* to convert these substances into thiamin by an *in vivo* synthesis.

¹³ In place of 6-hydroxy vitamin these experiments were actually carried out with the readily available "chloroxy vitamin" (Buchman, E. R., and Williams, R. R., *Jour. Amer. Chem. Soc.*, **57**, 1751 (1935)). From the results presented in this paper on the conversion of chloro thiazole to vitamin thiazole it may be inferred that chloroxy vitamin and 6-hydroxy vitamin are physiologically equivalent in work with the pea root.

¹⁴ Both acetopropyl alcohol and chloroacetopropyl alcohol were used in an attempt to demonstrate an *in vivo* synthesis.

¹⁵ Todd, A. R., and Bergel, F., *Jour. Chem. Soc. (London)* 1937, 364.

¹⁶ Buchman, E. R., *Jour. Amer. Chem. Soc.*, **58**, 1803 (1936).

¹⁷ The thioformamide is undoubtedly largely decomposed during the autoclaving of the medium (a procedure used in all of the experiments reported here). In accordance with this it was found that approximately 10 times as much of the mixture of thiazole intermediates as of vitamin thiazole must be used to support the growth of pea roots equally well. The *in vivo* synthesis of vitamin thiazole does not take place in the absence of added thioformamide, and hence either it itself or its decomposition products must enter into the reaction.

¹⁸ Buchman, E. R., and Richardson, E. M., unpublished.

¹⁹ The reasonable assumption is made that all substances, save only the vitamin itself, derived *in vivo* from these five thiazoles and vitamin pyrimidine, have *Phycomyces* activities comparable to those of the thiazoles when tested in a mixture with vitamin pyrimidines. See also reference 4.

²⁰ Williams, R. R., *Jour. Amer. Med. Assoc.*, **110**, 727 (1938).

CHROMOSOME PHYLOGENY

By C. E. McCLUNG

ZOOLOGICAL LABORATORY, UNIVERSITY OF PENNSYLVANIA

Communicated August 14, 1935

For a full century biologists have found the conception of the cell as a structural and functional unit an invaluable aid in the interpretation of vital phenomena. The fact that this unit is large enough for its parts to be seen in different functional stages has made the interpretation of structural conditions possible to a degree otherwise unattainable. Much remains to be done in this way, but for any striking advance new guides are required. Biologists join the physical scientists in the search for lesser units to explain operations within the cell. The fundamental question that then arises is this: Are these units merely those with which chemists and physicists are accustomed to deal, or are there others, characteristic only of living systems? Would a complete understanding of atomic and molecular phenomena be sufficient to explain vital phenomena or would there still remain for solution by the methods of biology a residuum characteristic only of living things? Most biologists are of the opinion that there is a field characteristically their own and this view has been more clearly defined during the years of the present century by the combined work of cytology and genetics. This has required for its elaboration the postulate of conceptual submicroscopic units or genes, which stand for living phenomena in somewhat the same relation that atoms and molecules do for the non-living. The philosophical necessity for such aids to the understanding of organic processes has always existed, and has frequently been supplied by theorists seeking to explain particularly the processes of inheritance, but it is only in recent years that the mechanisms involved have been so well understood that quantitative measurements could be applied. The existence of biological units is now quite as well demonstrated as is that of physical units, and their position in the cell is known, not merely in general, but individually, in cases sufficiently studied.

It is now quite apparent also that the cell is composed of a racial portion, the nucleus and another more directly involved in the processes of the individual, the cytosome. Within the nucleus, particularly apparent in the germ cells during all their changes, are found the chromosomes, which in their structure and behavior supply the mechanism required to explain genetic phenomena. Extensive, varied and consistent studies have shown that somatic characters result from the functioning of specific controls, or genes, within a reaction system; that these genes are aggregated into chromosomes of constant and specific character, which in their size and number correspond to groups of somatic characters; that the order of ar-

rangement of the ultimate determiners within the chromosomes is linear and generally constant; that disturbances of the order of the determiners is followed by changes in their operation; that certain regions of chromosomes do not have apparent direct relations with particular ultimate somatic characters as do the others; that specific effects are produced at different times in development; that individual organisms of great complexity pass through the same developmental stages as do those of simpler organization and then proceed further into distinctive changes of their own; that all organisms, whether simple or complex, are dependent for their existence upon the performance of a similar, limited series of functions; that during development there is a definite correlation between the character of the performance of these functions and the state of complexity of the structures involved. These conditions must depend upon the organization of the cell in relation to the determining racial elements which govern its operations. In other words, the problem is to explain present performances in terms of past experiences, recorded in the continuous, racial elements.

The importance of the linear order of the elements of the chromosomes is clearly indicated by its constancy and by its prominence and evident importance in relation to mitosis. The whole series of complicated phenomena in karyokinesis is obviously concerned in the maintenance, from generation to generation, of the precise linear organization of the individual chromosomes. It was the recognition of the importance of this fact which brought to Roux his conception of the theoretical significance of the chromosomes. There is nothing in all the structure of the cell more precise and constant, which indicates that there is nothing more significant.

Certain questions, naturally, arise: How did this linear order of the genes become established and what significance does it have ontogenetically and phylogenetically? In answering these questions we have to recall the all-important fact that living things are always in a state of becoming something else. In individual development a one-celled individual becomes in a short time an organized aggregate of innumerable cells. A species, over a longer period of time, becomes of another type. The whole system of organic forms is regarded as advancing from relatively simple conditions to more and more complex—there is a prevailing direction to the constant and endless variation everywhere apparent. This involves an increase in the number of parts and a consequent greater complexity of relations between them. If this is true of the visible structures which constitute organisms, it must also be true of the controls upon which these depend for their existence. What comes out of an egg depends upon what goes into it—and what goes into it are the results of the accumulated experiences of its innumerable forbears. We cannot but assume thus there has been a constant increase in the number of genes corresponding to the greater complexity of higher organic forms. Since additional parts, in passing from

relatively simple to complex, are added according to a prevailing pattern, we are forced to conclude that additional genes must take their place conformably in the system.

How do new genes arise and how are they added to the system? It would undoubtedly be most satisfying to physicists to know how electrons, atoms and molecules came into existence, but even without this knowledge they are able to make progress in their understanding by using these hypothetical concepts as guides to their thinking. The biologists are similarly situated with respect to their ultimate conceptual units. Undoubtedly in every case these origins are not isolated and independent things but the results of interactions between conditions or systems, which have arisen in response to preëxisting conditions. We may assume that the first living particle—let us call it a gene—arose in direct response to surrounding conditions. These conditions we know must have been those of the narrowly limited range which present living things require. The gene must have had, because of its form of organization, those properties which characterize the living system—preëminently the power to transform other substances into elements of its own system and the ability to reproduce similar units. It is no more teleological to make such an assumption than it is to conceive the properties of chlorine to be what they are because of the peculiar organization of the chlorine unit, or atom. Units have different properties because they differ in organization. One common characteristic of most units, however, is that they do not exist separately but are united into systems. We do not find genes as free and independent elements, but only as parts of larger organizations or chromosomes. Here they are distributed demonstrably in a linear order from proximal to distal. At the proximal end chromosomes are often directly related to the rest of the cell, and cannot maintain themselves in the system without this connection through the acromite.

Assuming that the beginning of a living system was a single unit, how did others become connected to it to form a chromosome eventually? there are, of course, two theoretical possibilities—either separate units come together, or the original unit, because of its power of reproduction, increased the number by dividing. The latter method is observed in every mitosis, but the products are identical and nothing new is added to the system. The reason for this obviously is that the plane of division is longitudinal and the series is merely duplicated. If, however, the hypothetical single genes had also the power, in response to the conditions under which the system must exist, to divide so that one derivative would be proximal, the other distal, e.g., to the substratum, then diversity instead of uniformity would result. Equivalent division is the almost exclusive form and must be consequent upon forces within the dividing unit; differential division is rare and presumably follows as a reaction between the

system and its environment. This seems to be a logical necessity for the system cannot exist apart from its environment and is constantly reacting with it. This gives a partial answer also to the very pertinent question: "Why, if the single unit system is able to maintain itself, should it become more complicated by the addition of more parts?" Undoubtedly by the very nature of a living unit, it must vary. In this respect it is just the opposite of non-living units, which by their inner nature must remain relatively constant. The explanation of one case is just as immediate as the other. The entire history of living things shows clearly also that this variation is in the main unidirectional—from simple to complex. This complexity may result from changes within the single cellular unit or by the addition of more units. Both of these methods are found in living systems, but obviously for the production of chromosomes additional units are required. If, then, we assume that the original single unit may divide so as to produce seriation as well as equivalent duplication, there is provided the means by which ultimately linear strings of genes, embodied in chromosomes, will result.

Under this assumption it follows also that the spatial order from proximal to distal, represents, in general, the temporal seriation. There is, however, no reason to suppose that this is necessarily absolute, because any unit in the chain might perform such a division if the proper stimulus were applied, but in general the older elements in the series would be proximal. If the assumption is correct that particular characters are due to the distinctive action of discrete units in a reaction system then it follows that greater complexity—more characters—means more control units, i.e., there must be more genes in more chromosomes. Such an assumption must, however, not be taken too narrowly. There is obviously not a single, one-to-one relation between a gene and a character. One gene has many effects, and probably, in some degree every gene in the system is involved in the production of each character. After all, the structures of an organism are significant only in relation to the functions they perform, and these common functions of all organisms are relatively few. Phylogenetic advance is not marked by additional functions but by the better and more highly developed performance of certain ones of the common series. Since so much of the value of an element in a reaction system is measured, not by its mere presence, but by the range and character of its interactions, the addition of a single element has effects far out of proportion to its numerical relation to the whole.

The time at which a factor operates has been shown experimentally to modify its distinctive effect. Sometimes if a factor fails in specific operation at its normal time, its customary effect on certain developing characters fails. The element of time is very significant in development. The question naturally arises therefore, "Is there any structure in the cell

which seems adapted to provide a temporal sequence of functioning controls? Particularly, since the controls, or genes, are located in the chromosomes, does their structure suggest a sequence in operation?" Very clearly the linear order of chromosome organization supplies the answer to this question. If sequence were not involved in the operation of controls the genes could just as well exist as separate elements. There is obviously great significance connected with the linear association of genes into chromosomes, and it seems most reasonable to conclude that this lies in the temporal sequence of their addition to the series and a resultant order of their operation in it. That this is true is indicated by the fact that inversions and translocations may be followed by distinctive genetical effects, i.e., the same series of controls in two different orders may produce two characteristically different results.

Most of the y-chromosome and certain portions of other chromosomes have been described as "inactive" or "genetically inert"—this because no somatic characters have been experimentally associated with definite regions in them. In a reaction system it is inconceivable that any part can be continuously present and without effect upon its operation. Its mere presence would modify the processes going on and the energy required for maintenance and reproduction must, by that much, lessen the total energy available in the system. It is very evident that there cannot be "inactive" chromatin in a cell, but it is equally clear that the customary association between definite chromosome levels and particular eventual characters cannot be made. What is the answer to this apparent paradox? Embryological characters, as a rule, have not been associated with individual genes. These result from processes of early somatogenesis and not the minute final differences usually employed to characterize gene action. It is entirely possible that these comprehensive effects, found in early stages of development, are due to the operation, principally, of these so-called inactive regions. This is entirely consistent with the assumptions previously made, for these changes occur early in the series and the "inactive" regions are proximal in position, where the assumed oldest genes occur. It is possible that any particular chromosome would show a different proportionate structure if studied at different ontogenetic stages. Somatic cells, representing the final changes in a differentiating series, might well exhibit such differences when compared with germ cells, particularly so far as genic structure is concerned. Some of the variations between the proportions of salivary gland chromosomes and those of the germ cells may be due to this cause.

The problem of why genes are associated into chromosomes seems to be reasonably solved by the assumption of sequence in accession and functioning; there remains the further problem of accounting for the presence of more than one chromosome in a nucleus. Sexual reproduction assures

at least two chromosomes in a somatic nucleus and this is apparently realized in *Ascaris megalocephala* var. *univalens*. Commonly there are more in a duplicate series. Whatever the number, often a single representative of each kind of chromosome, or a haploid set, is sufficient for normal development. The absence of a single kind of chromosome, or even the part of one, will prevent normal functioning. Obviously some form of chromosome interaction is involved, and this would suggest distinctive characteristics for each chromosome. Doubtless this differentiation exists, but the nature of it is not apparent. The extensive analytical genetic work on *Drosophila* does not indicate any regional effects. Factors for eye, body or wing characters may be found in two or more chromosomes. Lethal factors are similarly distributed.

Suggestive, of course, is the case of the sex-chromosomes. Here a part of one chromosome affects all the characters of the body, modifying them so that they take on alternative aspects in the two sexes. This has usually been regarded as a special case, but it is probable that in one way or another the action of genes commonly has this pervasive effect. It is clear enough that some minute distinctive character which is used to designate a gene cannot be the total of its effect in the system. A careful analysis of the data already obtained from *Drosophila* might do much to reveal the fundamental and systemic effects of genes. Work like that of Poulson ("Chromosomal Deficiencies and the Embryonic Development of *Drosophila melanogaster*," *Proc. Nat. Acad. Sci.*, 23, 133 (1937)) brings definite information regarding the profound influence of even a small portion of a chromosome upon development. Here a deficiency extending from 1.5 to 5.5 containing genes named for eye and wing characters, is sufficient to prevent almost entirely the formation of entoblast and mesoblast in the embryo.

It may not be without significance that the deficiency which prevents normal development in these early embryological characters is located in the proximal region of the chromosome. The relation of the proximal portions of chromosomes to early embryological characters may also be indicated by the fact that all the striations here are continuous through the chromocenter in *Drosophila*, suggesting homologies in genes and their effects in development (Prokofyeva-Belgovskya, *Cytologia*, 6, 442 (1934-35)). It is already apparent that in the endeavor to analyze the relations of genes to characters in development, we must not be misled by considering only their effects upon superficial characters.

If deficiencies in different regions of this or other chromosomes have their own distinctive effects, it would be possible to test theories of gene and chromosome action. Here again the sex-determining mechanism is suggestive, for it has been shown that the portion of the X-chromosome so concerned produces its effects only in coöperation with the remaining chro-

mosomes. In the reaction system of the cell, one chromosome establishes a balance in relation to the others which is determinative in shaping the characters of the body in one or another mold—all the characters, not just a few. This must be essentially what happens in the determination of all structural relations of the body. It is impossible to consider a sole and direct relation between one determiner and one character. This would mean lack of interrelations between determiners, something which does not exist, according to all the evidence we have.

The nature of this interrelation is a part of the general problem of cell organization. There is no understanding of the character of any element of a cell apart from its associations in the whole. While the final aim of cytology is a comprehension of this whole, our only hope of achieving it is through an analytical treatment of its constituent elements. At present our strongest hope is through a study of the chromosomes, but in thus concentrating upon them we do not lose sight of their place in the cell as a unit. Likewise, in an effort to understand the nature of a chromosome as a whole, we are forced to subject it to an analytical treatment. With our present understanding there is no hope of progress toward a comprehension of the functions of a chromosome in terms of the whole unit. Practically all that we know of the subject was learned by studying the effects of its parts, or genes, in reproduction. But the chromosome is a unit—there is something significant in this unit. In maturation and fertilization its own movements are important. To understand how a cell came into existence we will need to know how more than one chromosome enters into the formation of a nucleus and what is the nature of the resulting interrelations. In any aspect of the living system the basic element is that of interrelations, or organization, which comes about as a result of the nature of its constituent parts and it is not due to the action of external forces. It is only with full appreciation of this fact that we can hope to arrive at an understanding of the place and function of the chromosomes in the cell.

FEATHER CHARACTERIZATION AS STUDIED IN HOST-GRAFT COMBINATIONS BETWEEN CHICK EMBRYOS OF DIFFERENT BREEDS

By B. H. WILLIER AND MARY E. RAWLES

DEPARTMENT OF ZOOLOGY, THE UNIVERSITY OF ROCHESTER

Communicated August 18, 1938

In previous reports^{1,2}, it was shown that minute pieces of head skin ectoderm from chick embryos of the breeds Barred Plymouth Rock, Rhode Island Red and F₁ hybrid (Barred Plymouth Rock ♀ × Rhode Island Red ♂), grafted to the base of the wing bud of an embryo of the White Leghorn breed results in the formation of an extensive area of pigmented down feathers covering the entire wing and often adjacent parts of the breast, back and thigh. In reciprocal experiments, skin ectoderm from White Leghorn grafted to the pigmented hosts failed to produce patches of white feathers.

After hatching, the down feathers of such areas are gradually replaced by juvenile contour feathers having the form, rate of growth and arrangement in tracts characteristic of feathers in corresponding positions in host controls but always the color of the donor breed. In other words, the feather formed resembles the host feather in all respects except for color or color pattern which is similar to, if not identical with, that of the donor breed. The manner of origin of the donor-colored feather areas on the White Leghorn hosts remained problematical.

The purpose of the present paper is to make a further analysis of the rôle that both donor and host seem to play in feather characterization in the graft area. This involves an examination of two hypotheses as to the mode of origin of the donor-colored feather area. (1) That it arises solely by growth and spread of the original implanted piece. Such an origin could readily account for the result since the feathers of this area would be derived from feather germs,³ the epidermal component of which is composed of donor cells alone. (2) That it is formed from host skin but, owing to some influence from the implant, the feather produced becomes donor-colored. In this case, the implanted skin ectoderm, although incorporated at the implantation site, replaces little, if any, of the host epidermis of the graft area. Under such conditions the feather formed would be the product of the coöperation of host feather germs and of donor cells (chromatophores) or diffusible substances originating from them.

For this investigation, graft-host combinations between embryos of Barred Plymouth Rock, Rhode Island Red, F₁ hybrid (from the cross Barred Plymouth Rock ♀ × Rhode Island Red ♂), Black Minorca, Buff Minorca, S. C. White Leghorn, White Plymouth Rock, White Wyandotte

and White Silkie bantam breeds have been tested. Both skin ectoderm to which some mesenchyme adheres and pure limb bud mesoderm were used as implants. The source of the skin ectoderm was usually the dorsal-lateral surface of the head anterior to the otocyst but, in a few cases, it was taken from other regions of the embryo such as the wing, leg or back. The site of transplantation was usually the base of the wing bud; however, in some cases the transplant was placed on the dorsal surface of the head,

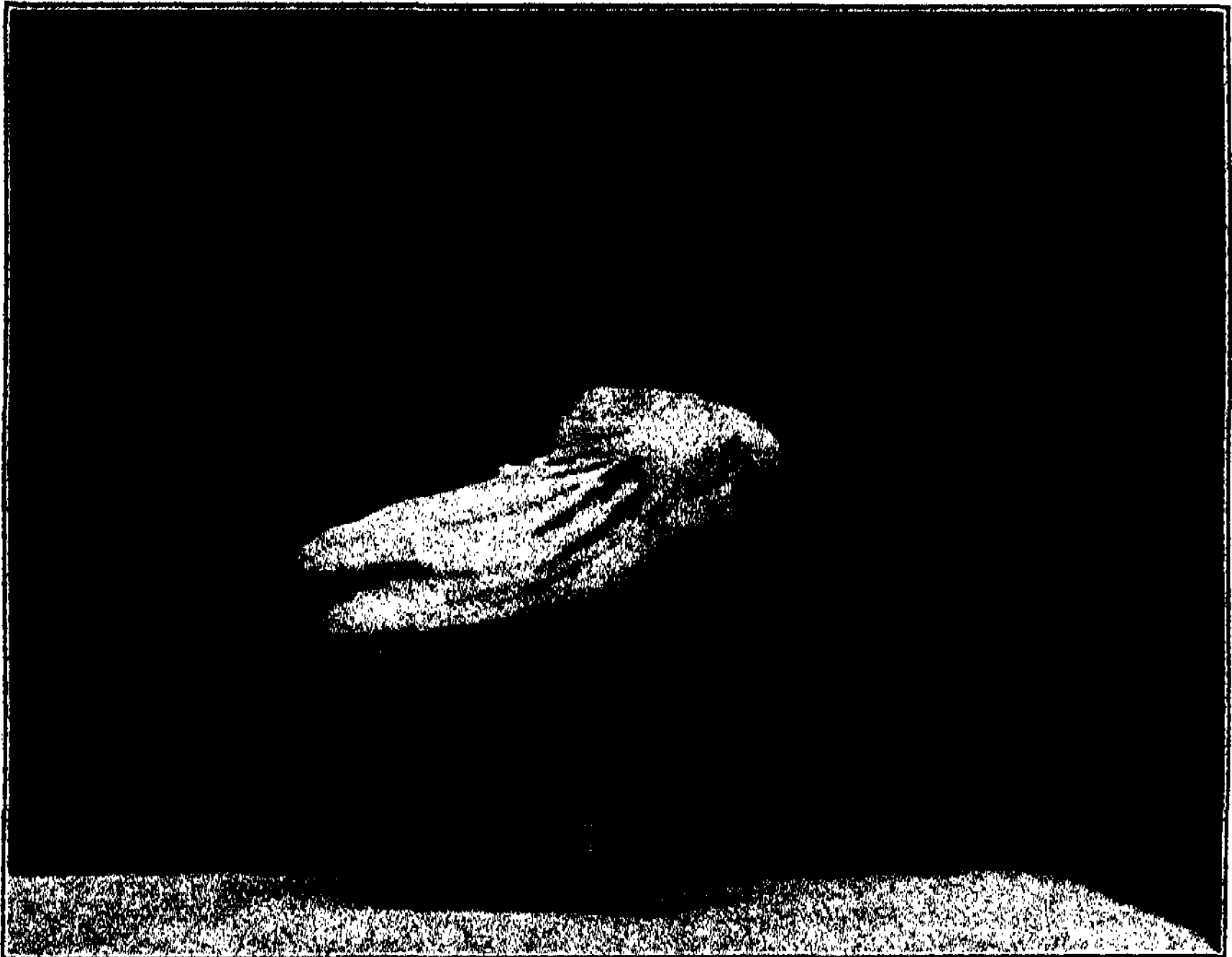


FIGURE 1

A 15-day old Black Minorca chick exhibiting white plumage on the wing and breast, produced by grafting to the limb-bud base of the host at 72 hours' incubation a piece of skin ectoderm from the head of a White Silkie embryo. The flight feathers, although donor-colored, are in structure, shape, rate of growth and distribution like host control feathers.

the hind limb bud or the tail bud. The method of grafting, age of embryo used as donor or host and subsequent treatment of the operated egg were the same as described in a former paper.¹

Skin Ectoderm Grafts.—Grafting a piece of head skin ectoderm from embryos of breeds with pigmented feathers or with white feathers to embryos of breeds having either white or pigmented plumage results in the formation of an area of donor-colored feathers on the wing and often adjacent regions of the host (Fig. 1). When White Leghorn, however, is

donor to Barred Plymouth Rock, F_1 hybrid and Rhode Island Red host embryos, no white feathers appear in the graft area. Black and Buff Minorca hosts are the only ones on which a White Leghorn implant has produced a patch of white feathers on the wing. Furthermore, it has been found that host-graft combinations of the various white-feathered breeds (Leghorn, Wyandotte and Rock) always produce white, not pigmented, feathers, donor and host feathers being indistinguishable.

When head skin is placed into the tail bud, the tail region of the chick embryo develops donor-colored feathers. Skin ectoderm obtained from other regions (wing, leg or back) of the embryo is likewise capable of producing a donor-colored feather area when transplanted to the wing bud base or to other sites such as the head, leg and tail. In these experiments on possible position effects, the host was usually White Leghorn and the donors Barred Plymouth Rock, Buff Minorca or F_1 hybrid embryos.

Irrespective of its source, skin ectoderm produces on the head a small localized area of donor-colored feathers instead of a large, much spread out patch which occurs when a transplant is placed into the wing, leg or tail buds. In general the path of extension of the effect is toward the tip of the limb and ventrally to the mid line of the breast when the implant is inserted into the base of the wing bud. The spread never extends dorsally to or across the mid-dorsal line. There is nearly always less spread when Barred Rock is host.

After hatching, the donor-colored down feathers of the graft area are gradually replaced by the juvenile plumage. The contour feathers of this plumage have the color or color pattern of the donor breed (Fig. 1). Moreover, when skin ectoderm from the F_1 hybrid embryo is grafted to White Leghorn hosts, sex-linked differences in color pattern of the juvenile plumage are found. Irrespective of the sex of the host, skin ectoderm from male and female donor embryos⁴ produces, respectively, barred and non-barred (solid black) contour feathers in the graft area. The male and female color patterns thus produced in the Leghorn resemble very closely the male and female patterns found in donor control chicks of the same age.

In some chicks the entire feather is donor-colored, in others partly donor- and partly host-colored. In feathers of the latter type the distal portion of the vane is the color of the donor and the proximal portion host-colored. The transition between these two portions of the vane is more or less sharp. In general the proportion of these portions varies with the sequence in origin of the feathers, the amount of the donor-colored portion being greatest in primaries and secondaries that arise first, and least in those arising later. The donor influence on color production which thus ceases before the emergence of the juvenile plumage is completed never reappears.

On the other hand, the feathers of the juvenile plumage which replaces

the donor-colored down have the form, rate of growth and arrangement in tracts characteristic of feathers in corresponding positions of host controls (Fig. 1). In no case do the feathers formed have any resemblance in shape or distribution to the feathers expected from the donor skin implant. The feathers of a graft area which covers the wing and adjacent parts of the breast, for instance, have the arrangement and form characteristic of primaries, secondaries, coverts and breast feathers, although the implant came from the head.

The daily rate of growth in length of certain primaries and secondaries has been measured and found to follow exactly that of the host on the unoperated side (left) or of host control. For example, remiges (flight feathers) having the color pattern of the donor Barred Rock, which is a slow-feathering breed, are identical in length with those of the left (control) wing of White Leghorn host which is a fast-feathering breed. They greatly exceed the length of wing feathers of a normal barred control of the same age. Also, red wing feathers produced by the donor Rhode Island Red are slowed to the Barred Rock rate when the latter is host.

The donor-colored juvenile plumage of the graft area is gradually replaced with adult plumage which is usually host- and not donor-colored. The molting of the remiges takes place in a very regular order as Warren and Gordon⁵ have described. The new adult remiges emerge in the same order in which the juvenile ones are dropped or plucked but with the color of the host. In certain exceptional cases some of the primaries or secondaries may be replaced with an adult feather which is a mosaic of donor- and host-colored areas of barbs. When sexual maturity is reached these molt and are replaced by host-colored feathers. Thus ultimately the donor-colored feathers are completely replaced with host-colored feathers.

Implants of Limb Bud Mesoderm.—Recent experiments of Mr. Ray Watterson, working in this laboratory, show that implants of a small piece of limb bud mesoderm alone from a Barred Plymouth Rock embryo (92–99 hours) introduced into the wing bud of White Leghorn host embryos (72 hours) produce an area of donor-colored down feathers having the same distribution and spread as skin ectoderm grafts produce.⁶ Upon hatching, the black down feathers are replaced by juvenile contour feathers having the barring pattern of donor control chicks but with the shape, rate of growth and arrangement in tracts characteristic of host controls (Fig. 2). Also he has found that if the entire limb bud mesoderm is freed of overlying ectoderm and inserted beneath the ectoderm of the host just behind the wing bud, it produces a stump-like process covered with donor-colored down feathers. Wing bud mesoderm of the White Leghorn grafted to a Barred Plymouth Rock host likewise gives an extra wing stump but the feathers covering it are host-colored, i.e., black, rather than white like the donor.

Manner of Origin of Donor-Colored Feather Area.—It is apparent from the data that both donor and host play a rôle in feather characterization within the graft area. Several lines of evidence indicate that structurally the feathers of this area are of host epidermal origin. (1) The spread of the effect to include the wing and adjacent feather tracts on the breast, back and thigh is too extensive to regard the implant of skin ectoderm as the entire source of the epidermal cells of the feather germs. (2) An histologi-



FIGURE 2

A 9-day old White Leghorn chick showing barred plumage on the right wing, produced by grafting limb-bud mesoderm from a Barred Plymouth Rock embryo into the wing bud of the host at 72 hours' incubation (from Watterson).

cal study of the skin ectoderm implant, made at successive intervals after implantation, shows that it does not replace the host epidermis of the developing wing and adjacent regions but remains localized at the site of grafting. Its surface portion heals in, connecting with the surrounding host epidermis while the deeper portions (inserted into the mesoderm for anchoring purposes at the time of grafting) become disorganized and the cells intermingle with and become indistinguishable from the mesodermal cells of the wing bud. (3) Implanting Silkie bantam skin ectoderm to

Black Minorca or to Barred Plymouth Rock produces structurally normal contour feathers of the same shape, rate of growth and distribution as those of the host and not feathers with missing barbicels (a characteristic of Silkie feathers) as would be expected if the donor epidermis produced them. (4) Implants of limb-bud mesoderm alone can produce donor-colored feathers. In this case, the epidermis of the host feather germ is undoubtedly concerned in the formation of the feather structure.

On the basis of these findings, the interpretation is reached that the feather of the graft area is the product of the joint action of (a) host feather germs and (b) some influence originating from the implant. The feather structure is the product of host feather germs but in some way its color or color pattern is produced under the control of the implanted cells. Whether this control is mediated through the action of donor cells (chromatophores) which migrate into the epidermal "collar" of the host feather germ or of diffusible substances released from donor cells situated in the dermal papilla of the feather germ and the mechanisms involved remain for future elucidation.

Control of Feather Color by White Leghorn Implants.—Implanting a piece of White Leghorn skin ectoderm (or mesoderm in some Barred Rock combinations) produces a donor-colored feather area in Buff and Black Minorca hosts but not in hosts of the Barred Rock, R. I. Red or F₁ hybrid breeds. With respect to feather color production the pigmented breeds tested fall into two classes. In hosts of the Minorca breeds the donor feather color of the Leghorn is expressed, thus following the rule found to hold for all other donor-host combinations tested. In the Barred Rock-R. I. Red breeds, however, the host suppresses in some manner the White Leghorn control of feather coloration. This phenomenon is well brought out by transplanting a White Leghorn limb bud with intact ectoderm to hosts of these breeds.¹ This results in the formation of a normal limb except for a covering of black down feathers. From this it is apparent that although the feathers arise from donor feather germs, their black color is produced under the control of the host. Whether this is an activation of potential melanophores in the grafted limb by some influence from the host (cf. DuShane⁷) or the result of an invasion of host melanophores is not evident from the data. In any case, the controlling factors reside in the skin of the host and are not blood-borne substances. This is shown by the result that a portion of a limb bud of a White Leghorn embryo produces only white feathers in grafts made to the chorio-allantois of either Barred Rock or F₁ hybrid hosts.

¹ Willier, B. H., Rawles, Mary E., and Hadorn, E., *Proc. Nat. Acad. Sci.*, 23, 542-546 (1937).

² Willier, B. H., and Rawles, Mary E., *Anat. Rec.*, 70, Sup. 3, 81-82 (1938).

³ For the development of the feather germ the reader is referred to Lillie, F. R., and Juhn, Mary, *Physiol. Zool.*, 5, 124-184 (1932).

⁴ After removing the skin ectoderm, the donor embryo is allowed to develop until the tenth day or later when its sex is ascertained.

⁵ Warren, D. C., and Gordon, C. D., *Jour. Agri. Res.*, 51, 459-470 (1935).

⁶ For a similar effect produced by neural crest, see Dorris, Frances, *Anat. Rec.*, 70, Sup. 3, 91 (1938).

⁷ DuShane, G. P., *Jour. Exptl. Zool.*, 72, 1-31 (1935).

CONCERNING THE ORIGIN OF THE POLYTENE CHROMOSOMES OF DIPTERA

BY KENNETH W. COOPER

DEPARTMENTS OF ZOOLOGY, UNIVERSITY OF ROCHESTER AND COLUMBIA UNIVERSITY

Communicated August 26, 1938

In view of the unprecedented rapidity with which new findings on polytene¹ chromosomes are being published, it may not be amiss to draw attention to certain aspects which are not very often considered. Polytene chromosomes are known to occur in many larval tissues of Diptera other than those of the salivary gland. They have been recorded in the nuclei of the fat bodies, hypodermis, intestine, absorbing cells of the midgut, muscles, malpighian tubules, tracheal cells and sporadic cells in the brain (Balbiani 1881, Carnoy 1884, Dawydoff 1930, Heitz and Bauer 1933, Geitler 1933a, etc.).²

The writer has found that *Drosophila* larvae, fixed in alcohol, sectioned and stained with Heidenhain's haematoxylin, not only show unmistakable polytene chromosomes in the nuclei of the cells of the salivary glands and malpighian tubules, but frequently carry them also in the fat bodies, gut, hypodermis, some muscle fibers and oenocytes. Indeed, it appears that the large nuclei of those cells fated to histolyze during pupational reorganization all contain polytene chromosomes.³ Dawydoff (1930) has indicated that the occurrence of such polytene chromosomes is hardly to be accounted for by physiologic specialization associated with glandular activity, as Darlington (1937) and others have supposed.

Origin of Polytene Chromosomes.—In general, all insect larval tissues destined to undergo histolysis during metamorphosis appear to grow by an increase in the size of their cells rather than by cell division (Trager). In the Culicidae (Bogojawlensky, Trager, Berger), Muscidae (Pérez), Drosophilidae (Frolowa 1937, Poulson) and probably all other flies, virtually all of larval growth is effected by an increase in the size of the cells concerned rather than by an increase in their number.⁴ Buck (1937), Geitler (1934a,

1937a, 1938) and Berger¹³ have cytologically examined nuclei of cells which undergo such growth. They demonstrated that in these cases (Diptera and Heteroptera) polyploid nuclei⁵ are resultant, and the important point for the present purposes lies in the observed increase of chromosome materials during nuclear growth.

It is of considerable interest, therefore, to note that Buck (1937) has found that polytene chromosomes of the salivary gland of *Sciara* undergo a regular increase in dimensions correlated with growth of the nuclei. Furthermore, the largest somatic nuclei in the present writer's preparations have the largest polytene chromosomes, and the size of the contained chromosomes is proportional to the volume of the nucleus. The smallest tissue nuclei appear to be "resting" or *energetic*¹⁵ nuclei. That the polytene chromosomes are of different sizes among the nuclei of the tissues and that the largest polytene chromosomes occur in the largest nuclei was indeed noted by Balbiani (1881). Clearly, then, the polytene chromosome grows with the growth of its nucleus.

Koltzoff (1934), Bridges (1935), Bauer (1935) and many others have suggested that the polytene chromosomes of Diptera arise by repeated doubling of chromonemata, forming "multivalent chromosomes." Marshak¹⁸ has experimentally demonstrated such growth of the chromosomes by means of x-ray-produced deletions in the chromonemata bundle. Recent estimates of the number of strands composing the polytene chromosome of the salivary gland of late larval life all agree that the number is a large one (*scil.*—*Sciara*, about 300, Buck 1937; *Simulium*, 64–128, Painter and Griffen 1937; *Chironomus*, 350–400, Bauer 1936; *Drosophila*, 256–512, Hertwig 1935), whereas Nebel and Ruttle (1937) have presented reasons for believing that the mitotic telophase chromosomes of animals, as well as of plants, contain but four chromonemata. As no more than four chromonemata need be supposed to exist in the telophase mitotic chromosomes of Diptera, during larval growth there must be an enormous increase in the number of chromonemata in the chromosomes of polytene nuclei—an increase in chromosome materials.

As these polytene chromosomes occur in the greatly enlarged nuclei of the most varied tissues, similar conditions probably obtain throughout much of the growing larva. It seems likely that, considering the pertinent data, both the growth of the cells and the chromonemata increases are in some manner related. Consequently it is suggested that the nuclei of the growing larva repeatedly prepare for unrealized prophasic condensation. These preparations involve chromonematal duplications whose number is not diminished by subsequent mitosis. Whatever the impediment to mitosis may be, this block at a particular nuclear stage apparently is the cause of both larval growth by increase of cell size rather than cell number, and, in part, the formation of the polytene chromosome.

Following the last mitotic division of organ differentiation in the fly larva, a chromosome may be expected to exist in the nucleus in a relic coil (Darlington 1935a) of a fairly large number of gyres (viz., 12-16 for the *X* of *Drosophila melanogaster*). The chromosome probably would possess, as the work of Nebel and Ruttle (1937) suggests, but four visible chromonemata. However, during growth to the third instar there is an increase of possibly more than 300 chromonemata. This increase in the number of chromonemata or chromosome materials would have a marked effect upon the helix or relic coil of the chromosome.⁶

As the thread number of the chromosome is repeatedly increased by duplications of the component chromonemata, and as these chromonemata remain tightly bound together, the girth of the chromosome must increase. But the chromosome is of helical (not spiral) structure. As the girth of the line of a tight helix of given height is increased, so the number of gyres must be reduced. Briefly, the increase in the number, or size, or both, of closely bound and precisely juxtaposed chromonemata of the chromosome results in a force which will tend to uncoil the chromosome.

To this uncoiling force must be added still another, probably of chief importance. That a chromosome undergoing transformation to the polytene condition must grow in length has been pointed out by Heitz (1935), Metz and Lawrence²¹ and Bridges.¹⁶ Buck (1937), Painter and Griffen (1937) and Frolowa (1937)¹⁷ have, in fact, shown that such growth occurs in *Sciara*, *Simulium* and *Drosophila*, respectively. This is obvious, for the length of the lax polytene *X*-chromosome of *Drosophila melanogaster* is from 140-200 micra (Bridges, 1935), whereas the mitotic metaphase *X*-chromosome is little more than 2 micra in length. The latter length must be the height of the helix into which the chromonemata are coiled at mitotic metaphase. Thus it may be calculated that if the polytene chromosome length were equal to that of the uncoiled chromosome, the mitotic metaphase chromosome would possess either (a) an enormous number of coils (as Muller, 1935, conceived) far exceeding (over 200) the conditions in any known organism, or (b) the chromonemata must undergo multiple or double coiling (also, Muller, 1935) for which there exists no evidence for animal mitotic chromosomes.²³ Therefore it is suggested that the uncoiling or opening of the helix of the developing polytene chromosome is produced by both increase in length and number of its component chromonemata.

Buck (1937) and Bridges¹⁶ maintain that simple uncoiling of the "normal" chromosome will account for only about one-eighth to one-tenth of the length of the lax polytene chromosome of the salivary gland. The growth in length appears to be accounted for chiefly by expansion of intergenic regions low in nucleic acid content. The heterochromatic regions of the normal chromosomes, on the other hand, apparently undergo little

if any longitudinal growth in the formation of the polytene chromosome. Whether this is due to the dense accumulation of nucleic acids in the heterochromatic regions is problematic.

Pairing Forces of the Polytene Chromosomes.—The fact that polytene homologs are generally synapsed at the completion of their growth stages is possibly and even probably the result of at least two distinct phenomena, namely, the uncoiling of the chromosomes and the forces of somatic pairing.⁷ Somatic pairing is held to be due to the mutual attractions of homologous loci,⁸ and appears to be expressed to an extraordinary degree in the Diptera. Now chromosomes may be expected to synapse to the degree that homologous loci may be approximated to each other. In the closely coiled mitotic metaphase chromosome the number of approximating loci would be at a minimum, and would little exceed the number of gyres of the metaphase helix. However, it may be expected that more and more homologous loci of a chromosome pair may be brought together by their mutual attractions as uncoiling proceeds. Thus, with uncoiling as complete as can occur within the confines of a nucleus, somatic pairing may reach its zenith and result in actual synapsis of the homologous chromosomes serially along their length⁹ (compare Beasely¹²). If this is true, the supposed relational coiling observed by Koller (1935) in the polytene chromosomes of the salivary gland is little more than the perpetuation of the residual twists in the uncoiling chromosomes at the time of their pairing.

It has been observed that homologous polytene chromosomes do not always synapse (Bauer, 1936b), or fail to synapse regionally (Geitler, 1934a). Darlington (1937), probably correctly, points out that failure of synapsis in some cases may be due to a delay in the approximation of homologs. In addition, Painter and Griffen (1937) have shown regional peculiarities in the polytene chromosomes of *Simulium* to be associated also with incomplete synapsis.

Darlington's Hypothesis of Pairing.—Darlington's precocity hypothesis was brought forth in support of his belief that chromosome pairing and synapsis at meiosis is, in part, dependent upon the univalency of the synapsing chromosomes. However, Nebel and Ruttle (1937), and others have advanced reasons for the belief that leptotene chromosomes are split at the time of synapsis. Furthermore, the intimate synapsis of the polytene chromosomes—multivalent in strand number—further stresses the fact that Darlington's view is by no means an established one (also, Beasely¹²). It has been shown by Geitler (1934a), Buck (1937) and Frolowa (1937)¹⁷ that each incipient polytene chromosome is composed of more than one strand at the onset of synapsis with its homologue, and Painter and Griffen (1937) lean to the same view. There seems no evidence for Darlington's contention that the chromonemata are associated closely in pairs in the polytene bundle (Painter and Griffen, 1937). Fur-

thermore, Painter (1934b) pointed out that, in the case of triploid *Drosophila*, three polytene chromosomes come together and synapse along their length in the same intimate fashion as do the polytene homologs of diploids. Lastly, Berger¹³ has presented evidence, adduced from his studies of multiple chromosome complexes of Culicinae, which he believes further emphasizes the insecurity of Darlington's hypothesis of pairing.

Quite possibly the essential condition prerequisite to chromosome synapsis is a high degree of uncoiling of the homologs.¹⁰ Such a degree of uncoiling is apparently the common property of all first meiocytes in which synaptic phenomena occur, and presumably the unique property of the chromosomes of larval Diptera among nuclei of somatic generations (Geitler²³).

Darlington (especially 1932, 1937) has accumulated an overwhelming amount of data demonstrating that in meiosis the pairing chromosomes associate by twos. It has been abundantly demonstrated that in polyploids the two by two association in synapsis holds. Thus, in a trivalent, one thread synapses for a distance with a second but further along its length may synapse with the third homolog, and for no extensive region do the three threads undergo triple synapsis. Rather than conclude from such data that the associations of chromosomes are by pairs, and that pairing (synapsis) occurs *because* the leptotene threads are unsplit, it certainly seems advisable not to lose sight of an alternative interpretation involving the organization of the chromosomes at meiosis. Inasmuch as polytene chromosomes undergo total synapsis in both diploid and triploid somatic cells despite the number of their component chromonemata, it seems not improbable that at meiosis, regardless of the number (possibly four) of the chromonemata of the leptotene threads, the synapsing chromosomes are bilateral in organization, i.e., constructed in such a manner that each chromosome possesses but one, limited, pairing surface.²⁴ The polytene chromosome, on the other hand, may be considered radially symmetrical with respect to its synaptic surfaces.

Concluding Remarks.—In the case of the multiple chromosome complexes of the Culicinae, most recently studied by Berger^{13,14}, it would seem that only the initial stages (viz., polyploidy) towards the formation of polytene chromosomes are undergone. As the chromosome threads do not undertake marked growth in length, it is not surprising that they give no evidence of achromatic banding. Furthermore, it appears not unlikely that the increase in strand number effects an uncoiling of the relic coils of the telophase chromosomes, and gives rise to a bundle of somatically paired, parallel, chromonemata. With onset of prophase, condensation of the chromonemata from the bundles of straightened chromosome threads would result in a bunched association of free homologs (i.e., multiple chromosome complexes) similar to those described by Berger.

Bauer¹¹ has recently shown that the nuclei of the nurse cells of *Lucilia*, *Pollenia* and *Musca* become polyploid concomitant with pronounced nuclear growth. Polyploidy of these nurse cells is expressed by the formation of an haploid number of banded, polytene-like chromosomes. Bauer holds that these giant chromosomes later dissociate into many small chromosomes, apparently by condensation (helicization) of the chromonemata and consequent separation from the bundle.

Apparently the multiple chromosome complex and Bauer's nurse-cell chromosome represent two different stages in the evolution of the polytene chromosome. In the Culicinae the telophase chromosomes are uncoiled, but, in the absence of marked longitudinal growth, no polytene chromosome is produced. In Bauer's case the chromosomes similarly are uncoiled, but in addition they have also undergone marked longitudinal growth. Identity with the polytene chromosome is not attained by the nurse-cell chromosome for condensation of its component chromonemata sets in before full polytene differentiation has taken place.

The question naturally occurs why polytene chromosomes do not arise elsewhere than in Diptera when nuclear growth occurs with chromosome multiplication but without concomitant mitotic activity. Carnoy (1884), it is true, has recorded or figured polytene-like chromosomes in certain nuclei of Coleoptera, Hymenoptera, Neuroptera, Odonata and the pedal ganglion of *Arion*, but his observations have failed to receive confirmation by recent workers. One factor that seems generally absent outside of the Diptera is the tendency towards somatic pairing (Geitler, 1938). Not only this, but it is suggested that the block to mitotic division in cases of the *Gerris* type (Geitler, 1937, 1938) may occur at a stage after prophasic coiling has set in. For example, the block may occur at premetaphase and the chromatids merely separate. Or, on the other hand, the same end would be attained by a block prior to prophase as in the case of the Culicinae. Thus the nucleus would remain intact as it internally ascends the scale of polyploidy, but would not give rise to polytene chromosomes.

In conclusion I wish to extend my sincere thanks to Professor Franz Schrader, Mr. Arthur Steinberg and especially to Dr. John B. Buck, for much helpful advice and criticism.

¹ Darlington (1937) has employed this useful designation which is adopted in this paper. Koller (1935) coined the term, but stated that he did not propose to use it, preferring "multiple threads." The expression "salivary chromosome" has little to commend it.

² Consult the bibliography of Geitler's "Chromosomenbau," *Protoplasma Monographien*, 14 (1938), for references given by date.

³ Wigglesworth (*Insect Physiology*, 1934) cites Pérez⁴ as authority that "even in the extreme case of Diptera, many larval organs (Malpighian tubes; certain muscle groups) are remodelled [during metamorphosis] without much change to form those of the adult."

This suggests that polytene nuclei may be perpetuated into imaginal life in the cases of certain Diptera, viz., *Calliphora*; see also Bauer.¹¹

⁴ Berger,¹² Bogojawlensky, *Zeit. Zellforsch.*, 22, 47 (1934); Pérez, *Arch. Zool. Exp. Gén.*, 5 Sér., 4, 1 (1910); Poulson, *Exp. Génétique*, 3, 1 (1937); and Trager, *Jour. Exp. Zool.*, 76, 467 (1937).

⁵ In that the haploid number of chromosomes or chromonemata bundles are potentially capable of giving rise to a polyploid number of chromosomes (as in Bauer's¹¹ case), the polytene nuclei are polyploid.

⁶ The type of chromosome growth as must be conceived by Metz and his supporters will have the same general effects as here argued.

⁷ Somatic pairing and somatic synapsis in part may be considered as different degrees of expression of the same phenomenon. Buck, Painter, Painter and Griffen, Dobzhansky and Tan, *et alii*, have drawn similar distinctions.

⁸ Metz¹⁹ seems among the first who have pointed this out.

⁹ These considerations lead one to suspect that in Diptera the degree of somatic pairing at metaphase may indeed be dependent upon the extent of effective uncoiling at the preceding prophase. Note especially the observations of Metz^{19,20} that somatic pairing at metaphase is in turn due to an intimate association in the prophase when chromosomes are drawn out into long threads. Intensity of somatic pairing, Metz²⁰ found, decreased from early prophase to metaphase.

¹⁰ Nebel and Ruttle (1937) have suggested that extensive elongation of the first meocyte prophase chromosomes is an important condition prerequisite to synapsis of homologs. Beasley¹² holds that an increase in nuclear size and a lengthening of the duration of prophase are important factors in meiosis, as they allow complete uncoiling of the chromosomes.

¹¹ Bauer, H., *Naturwiss.*, 26, 77 (1938).

¹² Beasley, J. O., *Bot. Gaz.*, 99, 865 (1938).

¹³ Berger, C. A., *Carnegie Contr. Embr.*, 167, 211 (1938).

¹⁴ Berger, C. A., *Nature*, 141, 834 (1938).

¹⁵ Berrill, N. J., and Huskins, C. L., *Amer. Nat.*, 70, 257 (1936).

¹⁶ Bridges, C. B., *Jour. Hered.*, 29, 11 (1938).

¹⁷ Frolowa, S. L., *Nature*, 141, 1014 (1938).

¹⁸ Marshak, A., *Amer. Nat.*, 70, 181 (1936).

¹⁹ Metz, C. W., *Jour. Exp. Zool.*, 21, 213 (1916).

²⁰ Metz, C. W., *Biol. Bull.*, 43, 369 (1922).

²¹ Metz, C. W., and Lawrence, E. G., *Quart. Rev. Biol.*, 12, 135 (1937).

²² Geitler, L., *Protoplasma Monographien*, 14, 1 (1938).

²³ Geitler²² has similarly criticized Muller's views.

²⁴ Limitation of the expression of synaptic forces of genes to a single surface at meiosis might be due to an incomplete envelope of insulating or damping materials about the genes,

*SOME EFFECTS OF LIGHT INTENSITY AND SHADE OF BACKGROUND UPON THE MELANIN CONTENT OF GAMBUSIA*¹

By F. B. SUMNER AND P. DOUDOROFF

SCRIPPS INSTITUTION OF OCEANOGRAPHY

Communicated August 22, 1938

During the past year, the authors have continued their studies of the influence of optic stimuli upon pigmentation in fishes.² Two experiments have been performed upon the "mosquito fish" (*Gambusia affinis*). The first of these was designed to test the quantitative effects of backgrounds of various albedos upon the amount of melanin present in fishes which had been subjected to these for a considerable period. In the second series, the intensity of the incident light was the variable chiefly under consideration, though two different backgrounds were used with each degree of illumination.

In the first of these experiments, 40 female individuals, averaging (at the close of the experiment) 0.52 gm. in weight, were placed in each of 15 large glass bowls, half filled with fresh water. Care was taken that the average size of the fishes in these various lots was nearly equal.

The bowls were painted on the outside in five different shades: black, white and three shades of gray. There were thus three bowls of each sort. Counting the albedo of the white bowls as unity, the three grays rated as 0.33, 0.19 and 0.12, respectively, that of the black being (theoretically) 0.³ The bowls were kept in several nearly light-proof cabinets, each lighted overhead by a 100-watt electric lamp. The intensity of the light, as determined with a Weston "Foot-Candle Meter," was about 30 foot-candles at the level of the platform supporting the bowls. The water temperatures in the various bowls averaged about 23°C., and did not differ significantly from one bowl to the other. The fishes were fed three times weekly, partly with *Daphnia*, partly with a prepared food. In order that these artificial backgrounds should be obscured as little as possible, no plants were placed in the bowls. Likewise the latter were kept as clean as possible.

The fishes were exposed to these conditions for 69 to 71 days, when the survivors were killed for melanin determinations.

In our second experiment, only two shades of paint were used upon the bowls: black and the "medium gray" of the first experiment. Four bowls (two painted each of these shades) were placed in each of four cabinets. These last were very differently illuminated, the lamps used being respectively of 200 watts, 40 watts, 10 watts and 7.5 watts capacity, the last being additionally dimmed by covering with a translucent globe of white glass. The intensities of illumination, at the level of the platform, were, roughly, 90, 10, 1.5 and 0.25 foot-candles, respectively.

Rather close uniformity of temperature among the cabinets (within 0.4° or 0.5°) was secured by immersing the bowls in running fresh water. Throughout the experiment, the temperatures ranged between 22° and 23° (+).

Thirty-two females were placed in each bowl, again selected with reference to uniformity of average size. They were subjected to these conditions for 62 to 63 days. The mean weight in the present series was 0.74 gm. at the close of the experiment.

A considerable number of fishes employed in each of these experiments succumbed to an apparently infectious disease, which will be discussed in another paper. The numbers were not, however, seriously reduced, and the survivors (at least those used for the melanin tests) appeared to be perfectly normal.

At the ends of the periods stated, the fishes were killed and subjected to treatment, preparatory to the evaluation of their melanin. The steps in this treatment were largely those enumerated in our preceding paper.⁴ There were, however, certain alterations or abridgments of our earlier procedure. (1) The fishes, after killing, were dried for two days at 100° , instead of being placed in alcohol; (2) digestion with pepsin was omitted; (3) the magnesium carbonate procedure was omitted, the colorimetric readings being made directly upon the final solutions in NaOH. These last two time-saving omissions were adopted after we had found that the use of pepsin was superfluous, and that, with the species here used, solutions clear enough for direct colorimetric measurements could be prepared.

Despite laborious efforts, our hopes of obtaining quantitative results of satisfactory precision were not realized. Altogether unexplained differences of considerable magnitude appeared among samples of material which should have yielded identical melanin values. One reason for this was the fact, which we did not at first sufficiently realize, that even solutions of supposedly pure melanin are far from being stable in respect to color characters. They fade as a result of boiling or merely with the lapse of time. Later colorimetric readings of the same solution,⁵ even when kept in the dark, invariably gave lower values than earlier ones. That this circumstance affected both the "standards" (melanin solutions of known concentration)⁶ and the samples to be tested goes without saying.

Nevertheless, certain "qualitative" (i.e., only roughly quantitative) results were obtained and these seem worth reporting briefly. Further studies are now under way with *Lebistes*, in which the counting of melano-phores will be substituted for the measurement of melanin.

Considering these experiments in the order indicated, there were, as stated above, three bowls of each shade. Thus, there were three complete series of the fishes, and these were dealt with separately. The determinations were made by comparing each of the sample solutions in question

with a melanin solution of known concentration in a Duboscq colorimeter. The readings were interpreted with the aid of a curve, based upon dilutions of the "standard."

Figure 1 portrays the results obtained from this experiment. The complete curves, including that for the means, are based upon the first two of the series of fishes. Owing to a careless blunder in our procedure⁷ the readings for the "medium gray" and "dark gray" fishes of the third series are not available.

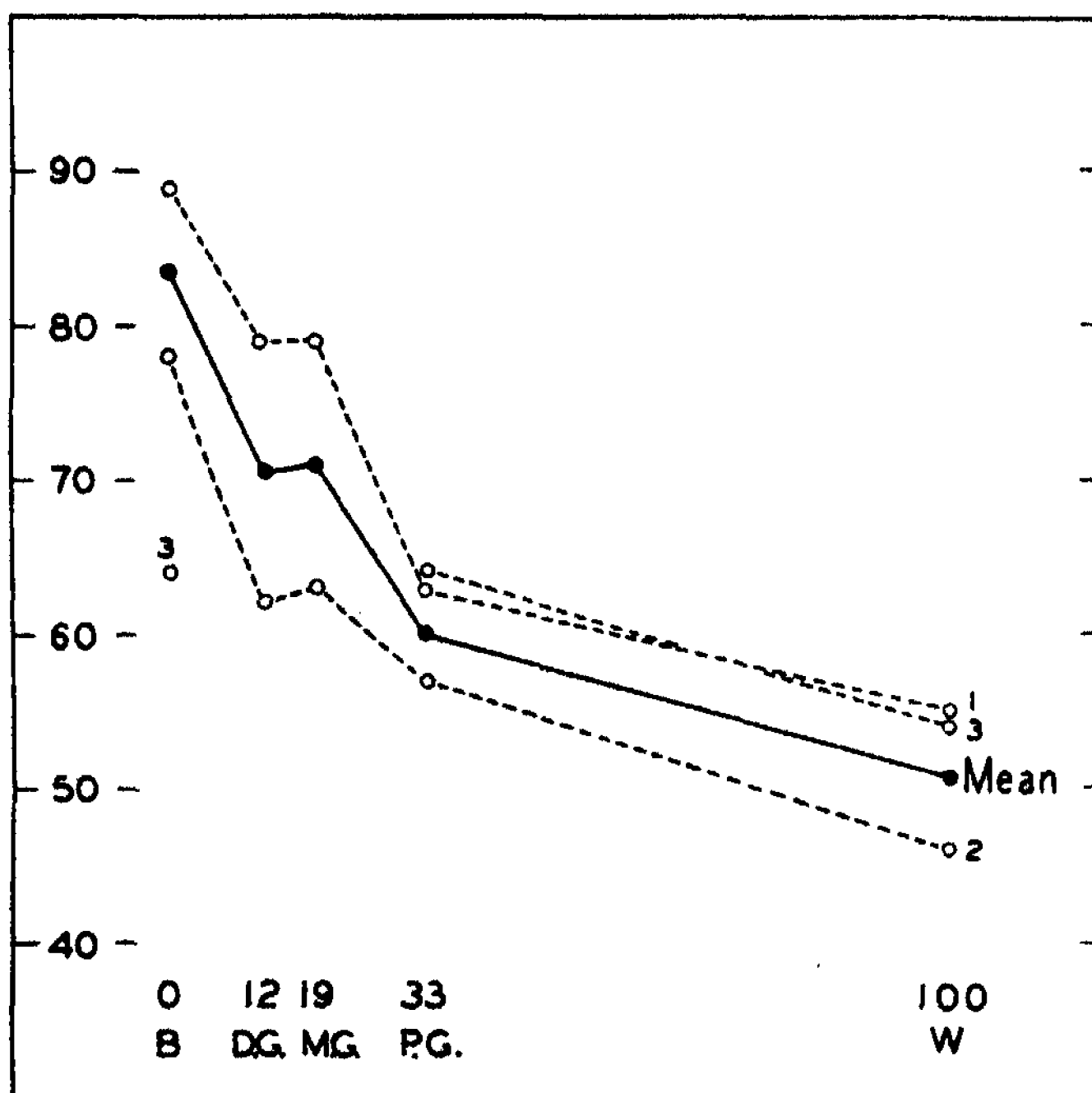


FIGURE 1

Computed melanin content of fishes exposed for 2 months (+) to backgrounds of various albedos. Abscissas represent albedos; ordinates the melanin content, expressed in milligrams per 100 grams of original weight of fishes. The "mean" curve is based upon the means for series 1 and 2.

Despite the fortuitous character of some of the differences, the trend shown in these graphs agrees fairly well with that shown in figure 3 of our earlier paper. The disconcerting features are (1) the extremely low position of the "black" figure in series 3, and (2) the virtual identity (slight reversal) of the figures for medium gray and dark gray.⁸ It will be seen, however, that the difference between the albedos of these two backgrounds was small.

TABLE 1

	BLACK		GRAY	
	Bowl 1	Bowl 2	Bowl 1	Bowl 2
0.25 Foot-candle	84	88
1.5 Foot-candle	94	84	77	86
10.0 Foot-candle	91	102	85	84
90.00 Foot-candle	98	93	75	88
Means	93.7		83.4	

The results from experiment 2 are presented in table 1. As in the case of the graphs in figure 1, the figures represent milligrams of melanin per 100 grams of the original weight of the fishes. In the present case, readings were taken with the Ives Tint-Photometer, the solutions being placed in absorption cells.⁹ Each lot of the fishes comprised from 18 to 30 individuals. Unfortunately the two lots of "black" fishes in cabinet 1 (0.25 F. C.) were rendered unavailable by an accidental interchange of reagents at one step in the preparation.

From the figures in the table, it will be seen that the average melanin content of the "black" fishes was about 12 per cent higher than that of the medium gray ones, and that the significance of this difference has a rather high probability.¹⁰ It is of interest, too, that no correlation is manifest between the melanin content of the various lots of fishes and the intensity of the illumination to which they had been exposed. However, such a correlation, if low, might easily be concealed by the high variability of the individual lots.

This lack of any observable effect of large differences in illumination upon melanin content is not in full agreement with our previous observations upon gobies (op. cit.) which seemed to indicate that the intensity of incident light exerted an effect, though a minor one, upon the amount of melanin present.

The far higher apparent melanin content indicated by table 1 for the fishes of the second experiment, in comparison with that indicated by figure 1 for those of the first experiment, depends, we believe, upon a difference in our procedure. The material derived from the former was not subjected to one step which was employed for the latter.¹¹

Summary.—Despite technical difficulties which resulted in considerable fortuitous variation in the indicated melanin values, we have been able to reveal for *Gambusia* the general correlation, which we had previously found in *Gillichthys*, between the melanin content of the fishes and the albedo of the background to which they had been subjected for some weeks. On the other hand, the absolute intensity of the incident light had little or no effect upon melanin content.

¹ Contributions from the Scripps Institution of Oceanography. New Series, No. 31.

² Cf. *Proc. Nat. Acad. Sci.*, 23, 211-219 (1937).

³ Reflections from the glass and debris on the bottom, though the latter was removed rather frequently, raised the albedo slightly at most times.

⁴ Op. cit.

⁵ The solutions were ordinarily prepared in 0.2% NaOH. However, this instability persisted after great reduction in alkalinity.

⁶ We employed melanin from the feathers of black Minorca fowls, kindly furnished us by Prof. R. A. Gortner.

⁷ The boiling in NaOH was left overnight, instead of being continued for three hours. We know that a material amount of fading results from such treatment.

⁸ The omitted values for series 3, which are strictly comparable *with one another*, show, however, a gradient in the expected direction.

⁹ Sumner and Fox, *Jour. Exptl. Zool.*, **66**, 263-301 (1933).

¹⁰ That is, 0.9978. For method of computing this, see Tippet, "The Methods of Statistics," 1931, pp. 80-82.

¹¹ This consisted of two successive final washes with alcohol, followed by ether, a procedure which certainly carried off traces of some colored substance, whether or not this contained melanin.

THE EFFECTS OF LIGHT AND DARK BACKGROUNDS UPON THE INCIDENCE OF A SEEMINGLY INFECTIOUS DISEASE IN FISHES¹

BY F. B. SUMNER AND P. DOUDOROFF

SCRIPPS INSTITUTION OF OCEANOGRAPHY

Communicated August 22, 1938

The facts here reported constitute a by-product of a research having quite other ends in view. In an immediately preceding paper we have discussed the nature of this research and presented such results as bear upon the main object.

The conditions imposed upon the fishes in each of these experiments did not appear to be altogether favorable to their health. Deaths occurred in nearly all of the bowls, the records of these being kept upon cards. Most of the fishes which died displayed similar symptoms, involving emaciation and lesions of the skin and fins, particularly of the tail. In advanced stages, the entire caudal fin and even portions of the fleshy part of the tail disintegrated. Unless subjected to salt-water treatment, the fishes usually died within a few days after the onset of the symptoms. This treatment was partially effective, and was doubtless responsible for the preservation of considerable numbers of those attacked. It consisted in immersing them in full-strength sea-water for an hour, then returning them to fresh water. The fishes in all of the fifteen bowls in the first experiment were thus treated once. Owing to the heavier incidence of the disease in the black bowls, these lots were subjected to two further treatments.

Throughout these experiments, the mode of handling the fishes was such that infection from any source was no more likely in the black bowls than in any of the others.

Our colleague, Dr. C. E. ZoBell, has been good enough to subject some of these diseased fishes to bacteriological examination and culture study. He reports that various organisms were found, but that one in particular was of constant occurrence. This he characterizes as a non-sporulating bacillus, Gram-negative, non-pigmented, non-motile, measuring from 2.0 to 3.8 μ in length, 0.6 to 0.8 μ in width, growing readily on nutrient fresh-water agar, but failing to grow on corresponding media prepared in sea water. Dr. ZoBell has further studies in progress, upon which he expects to report independently.

It was early evident that deaths were far more frequent in the black bowls than in any of the others. The figures for each type of bowl, at the end of the first experiment, were as follows:

TABLE 1

Black.....	43	(36%)
Dark gray.....	11	(9%)
Medium gray.....	2	(1.7%)
Pale gray.....	7	(6%)
White.....	3	(2.5%)

It is to be noted that nearly twice as many deaths occurred in the black bowls as in all the others combined. Of less certain significance is the fact that the number of deaths in the dark gray bowls is nearly equal to that in all the others, blacks excluded. In considering the preponderance of deaths among the "black" fishes, it must be recalled that these were subjected to the beneficial salt-water treatment more than any of the others. Had this not been the case, there would probably have been an even greater difference in morbidity between the "blacks" and the others.

For the second experiment we present the record of deaths in detail for each bowl of each cabinet. This record of "deaths" includes a few fishes which were badly diseased and were removed while still living. The onset of the disease was gradual. Only one death is recorded for the first eight days, and only two for the first fourteen days. The salt-water treatment was administered twice for all the lots.

TABLE 2

	BLACK		GRAY		TOTALS
	BOWL 1	BOWL 2	BOWL 1	BOWL 2	
0.25 Foot-candles	11	4	3	2	20
1.5 Foot-candles	8	11	5	4	28
10 Foot-candles	11	3	1	3	18
90 Foot-candles	12	8	6	6	32
Totals		68		30	98

In addition to the foregoing fishes, which were picked out dead or badly diseased, a considerable number of less diseased ones were rejected at the end of the experiment, when the fishes were killed for the melanin determinations. Of these, 14 specimens were from black bowls, 5 from gray ones. Thus 82 of the "black" fishes in this second series (32 per cent) died or showed evident symptoms of the disease, while only 35 "gray" specimens (13.7 per cent) did so.

From these two experiments, certain conclusions seem unavoidable.

No calculation of probabilities is needed to establish the fact that susceptibility to this disease was much higher among the fishes kept in black containers than among those kept in gray or white ones.²

Again, from the first series (table 1) it seems likely that fishes in dark gray bowls were more susceptible than ones in paler grays or in white.

An obvious suggestion will be that we have to do with some immediate effect of light or other radiant energy upon the parasitic organisms in the skin of the fishes. That no such conclusion is tenable is evident from several circumstances. In the first series, the incident light was identical for all, the only difference being in the light reflected from the walls of the bowls. In the second series, where the incident light varied widely, the mortality rate varied with the shade of the background, while light intensity played little or no part in the matter (table 2).

Nor can it be supposed that disease resistance depended in some way upon the "general health" of the fishes, which, in turn, was influenced by the light conditions in the bowls. If growth rate be regarded as indicative of health, it may be mentioned that in both of our series the surviving fishes in the black bowls were actually slightly heavier than those in any of the others.³

It seems plain that the incidence of this disease was in some way influenced by the same factors which were responsible for bringing about the color changes of the fishes upon the various backgrounds. Whether these differences in morbidity resulted from the changes of pigmentation *per se*, or from physiological processes underlying these last, we cannot say. But we will venture one suggestion.

It is now well known that a prolonged state of chromatophore "expansion" (properly of pigment dispersion within the chromatophores) is accompanied by an increase in the number and the pigment content of these cells, while prolonging the "contraction" (aggregation) phase of the chromatophores results in an elimination of many of the latter.

That these effects of optic stimuli, both the immediate and the more gradual ones, are due to the liberation of special hormones ("neurohumors") at the nerve terminals has been contended with a considerable show of evidence by a number of recent investigators, notably, in this country, by Parker and various collaborators. While the present writers lack any

direct evidence in the matter, we will suggest a possible interpretation of our results. May it not be that the differences in susceptibility to this disease among our various lots of fishes were the direct result of these hormonal differences rather than that they were due to differences in the amount of pigment present? Such an effect seems more readily attributable to physiologically active substances such as hormones than to relatively inactive substances such as melanin or xanthophyll.

We may imagine that the "expanding hormone," if such exists, is favorable to the infection of the skin by the specific organism involved, or that the "contracting hormone" may have a bactericidal effect in relation to the latter; or perhaps both of these suppositions may be true. However, without further evidence, any such suggestions are admittedly little more than guesses. We hope to conduct experiments along these lines in the future.

Summary.—The incidence of this disease was very much higher among fishes kept in black bowls than in ones kept in white or gray ones. It was apparently somewhat higher in dark gray bowls than in pale gray or white ones. These facts cannot be explained as the result of any direct effect of light upon the parasitic organism presumed to be responsible for the disease. They probably result from the same agencies which bring about the dispersed or condensed condition of the melanin under the influence of optic stimuli. It is suggested that diffusion hormones of the "neuro-humoral" type may be the agents involved.

¹ Contributions from The Scripps Institution of Oceanography. New Series, No. 32.

² Let us repeat that the paint was applied to the *outside* of the bowls.

³ This is perhaps due to the fact, that, being less numerous, the former obtained somewhat more food apiece. The bowls received approximately equal rations without reference to numbers.

THEORY OF TRANSVERSAL CURVES AND THE CONNECTIONS BETWEEN THE CALCULUS OF VARIATIONS AND THE THEORY OF PARTIAL DIFFERENTIAL EQUATIONS

BY M. HERZBERGER

KODAK RESEARCH LABORATORIES,* ROCHESTER, N. Y.

Communicated August 22, 1938

I. *Introduction.*—The theory of transversal curves endeavors to give the geometric equivalent to conceptions developed by Lagrange, Hamilton, Jacobi, Grassmann, Bruns and Poincaré with respect to partial differential equations, the calculus of variations and integral invariants. The calculus

of variations is taken, in the sense of its applications in physics, as the theory of the geometric qualities of manifolds of extremals, irrespective of whether they are maxima or minima. This investigation deserves a little interest from the physicist, since it shows the connections between the fundamental conceptions of his theories.

Let us assume that to each line element in n -dimensional space a contravariant vector, n^i , is given such that n^i as a function of x_i and \dot{x}_i can be considered as two times continuous differentiable at any regular point.

Let us assume that we may order our line elements into a $2(n - 1)$ manifold of curves such that, for any reasonably selected two-dimensional manifold, the Lagrangean bracket remains constant along our curves, i.e.,

$$\frac{d}{ds} (\delta n^i \partial x_i - \delta x_i \partial n^i) = \frac{d}{ds} [\delta \partial] = 0. \quad (1)$$

A $2(n - 1)$ manifold of curves for which (1) is fulfilled identically is called a manifold of *transversal* curves.

Our defining equation allows the substitution

$$n^i \rightarrow x_i, \quad x_i \rightarrow -n^i. \quad (2)$$

This fundamental substitution is allowed in all our results (*First Duality Principle*).

Let us designate differentiation along our curve by dotting the particular letter. Equation (1) is equivalent to

$$\partial \dot{n}^i \delta x_i + \partial n^i \delta \dot{x}_i - \partial x_i \delta \dot{n}^i = 0. \quad (3)$$

Equation (3) can be considered as an integrability condition for two functions $a(x_i, \dot{x}_i)$ and $b(x_i, n^i)$ (and their duals) with the following defining equations:

$$\delta a = \dot{n}^i \delta x_i + n^i \delta \dot{x}_i = \frac{d}{ds} (\dot{n}^i \delta x_i) \quad (4)$$

$$\delta b = \dot{n}^i \delta x_i - \dot{x}_i \delta n^i.$$

We call a and b *generating* functions of our transversal curves. Since they are determined except for a constant, we might standardize them by asking

$$a - b = n^i \dot{x}_i. \quad (5)$$

Each one of the two functions, provided it is not constant, is sufficient to determine our problem. For a given $a(x_i, \dot{x}_i)$ we have

$$\begin{aligned}
 n^i &= \frac{\partial a}{\partial \dot{x}_i} \\
 \text{or} \quad \frac{d}{ds} \left(\frac{\partial a}{\partial \dot{x}_i} \right) &= \frac{\partial a}{\partial x_i} \\
 \dot{n}^i &= \frac{\partial a}{\partial x_i}
 \end{aligned} \tag{6}$$

Equations (6) are the Euler equations of the variation problem $\delta E = 0$ with

$$E = \int a(x_i, \dot{x}_i) ds. \tag{7}$$

They determine our transversal curves.

If $b(x_i, n^i)$ is given, (4) shows that

$$\begin{aligned}
 \frac{\partial b}{\partial x_i} &= n^i \\
 \frac{\partial b}{\partial n^i} &= -\dot{x}_i
 \end{aligned} \tag{8}$$

Equations (8) can be considered as the Lagrange-Charpit equations of the partial differential equation

$$\begin{aligned}
 b \left(x_i, \frac{\partial z}{\partial x_i} \right) &= 0 \\
 \text{with} \quad n^i &= \frac{\partial z}{\partial x_i}
 \end{aligned} \tag{9}$$

Equations (8) therefore determine our problem again.

II. *The Geometry of Transversal Curves.*—If we have a k -manifold of transversal curves $2 \leq k \leq 2(n-1)$ given by k parameters, u_1, \dots, u_k , we get $\binom{k}{2}$ differential invariants for every curve given by the Lagrange symbol, as follows

$$[u_p, u_q] = \frac{\partial n^i}{\partial u_p} \frac{\partial x_i}{\partial u_q} - \frac{\partial n^i}{\partial u_q} \frac{\partial x_i}{\partial u_p} \tag{10}$$

The Lagrange bracket follows the equations

$$\begin{aligned}
 [u_p, u_q] &= -[u_q, u_p] \\
 [u_p, u_p] &= 0.
 \end{aligned} \tag{11}$$

The bivector with the $\binom{k}{2}$ components $[u_p, u_q]_{p < q}$ (Grassmann) can be taken as characterizing our manifold. If it is zero, we call the manifold a normal manifold. It is zero for instance when our manifold consists of

curves originating from a point. Integration of (11) gives the integral invariant of our manifold:

$$\frac{d}{ds} \int \int \Sigma[u_\rho u_\sigma] du_\rho du_\sigma = 0. \quad (12)$$

If we intersect our manifold by two surfaces, we can integrate (1) and find that

$$[u_\rho u_\sigma]_1 = [u_\rho u_\sigma]_2. \quad (13)$$

Equation (13) permits, besides substitution given in (2), the substitution

$$n_1^i \rightarrow x_{1i}, \quad x_{1i} \rightarrow -n_1^i. \quad (14)$$

This combined with (2) has been called the tetrality principle by the author, and has been widely applied in optics.

If integration of (13) is possible, it leads to Hamilton's characteristic functions. Application of (13) to the manifold of all transversal curves and two intersecting surfaces, such that through any one point of the first and any one point of the second surface goes one, and only one, transversal curve, leads to Bruns' "eiconal" functions, a special case of Hamilton's characteristic functions. Bruns' "eiconal" depends only on the $2(n-1)$ coördinates of the intersection points.

We can coördinate to our problem a problem in the $2(n-1)$ space, whose points are the combined coördinates of our two intersection points, whereas a contravariant normal vector can be defined by

$$N^i = (n_1^i, -n_2^i). \quad (15)$$

The general problem of our transversal curves becomes therefore the investigation of normal systems in the $2(n-1)$ space.

III. *Partial Differential Equations and the Calculus of Variation in Homogeneous Form.*—We now investigate the special cases where one of our "generating functions" degenerates.

(a) If $n^i \delta x_i + n^i \delta x_i \equiv 0$, we can assume that there exists a function

$$a(x_i, x_i) \equiv 0. \quad (16)$$

At every point there exists a surface given by (16).

We obtain from (5) and (8)

$$b(x_i, n^i) = -n^i x_i = \frac{\partial b}{\partial n^i} n^i, \quad (17)$$

in which b is a first-order homogeneous function of n^i . That means that n^i is determined by our problem only for an arbitrary multiplicative scalar function of x_i . Only its direction, not its absolute value, has a geometrical meaning.

From (4) we derive

$$\begin{aligned}\frac{d}{ds}(n^i \delta x_i) &= 0, \\ \frac{d}{ds} b &= 0.\end{aligned}\tag{18}$$

That means we have a contact transformation in the sense of Lie, and b is constant along our curves.

We can consider our curve as the characteristic of the homogeneous partial differential problem:

$$\begin{aligned}b\left(x_i, \frac{\partial \phi}{\partial x_i}\right) &= 0 \\ d\phi &= \frac{\partial \phi}{\partial x_i} dx_i = n^i dx_i = 0,\end{aligned}\tag{19}$$

where n^i is a vector normal to the solution surfaces.

(b) If $n^i \delta x_i - x_i \delta n^i \equiv 0$, then at each point we have a surface

$$b(n^i, x_i) = 0.\tag{20}$$

This surface is called in optics the indicatrix.

We find that

$$a = n^i x_i = \frac{\partial a}{\partial x_i} x_i.\tag{21}$$

That means a is a function homogeneous in the first order in x_i , i.e., the problem does not depend on the curve parameter, s . We have

$$E = \int a ds = \int n^i x_i ds = \int n^i dx_i.\tag{22}$$

The variation problem given by $\delta E = 0$ leads to our transversal curves as extremals.

IV. *General Variation Problem and General Problem of Partial Differential Equations.*—(a) If we have given a partial differential equation

$$B\left(X_i, Z, \frac{\partial Z}{\partial x_i}\right) = 0,\tag{23}$$

we define the vectors

$$\begin{aligned}P^i &= \frac{\partial Z}{\partial X_i} \\ Y_i &= \frac{\partial B}{\partial P^i} \quad G^i = \frac{\partial B}{\partial X_i}.\end{aligned}\tag{24}$$

Let us introduce Z , as a new variable and look, not for a function

$$Z(X_i) = 0 \quad \text{with} \quad P^i = \frac{\partial Z}{\partial X_i} \quad (25)$$

but for a function

$$\phi(X_i, Z) = 0 \quad \text{with} \quad \frac{\partial \phi}{\partial X_i} = n^i, \quad \frac{\partial \phi}{\partial Z} = n^{r+1}. \quad (26)$$

We can then obtain from (23) an equation with one more variable:

$$b(x_i, n^i) = n^{r+1} B \left(X_i = x_i, Z = x_{r+1}, \frac{\partial Z}{\partial x_i} = -\frac{n^i}{n^{r+1}} \right) = 0. \quad (27)$$

If we investigate specially the solutions for $n^{r+1} = 0$, the solution of the problem stated in (27) is identical with the solution of the problem in (23). Equation (27) is homogeneous in the first order in n^i and therefore identical with the problem dealt with in III (a). Differentiation of (27) and comparison with (8) gives

$$\frac{\partial b}{\partial x_i} = \dot{n}^i = n^{r+1} \left(G^i, \frac{\partial B}{\partial Z} \right) \quad (28)$$

$$\frac{\partial b}{\partial n^i} = x_i = (-Y_i, A),$$

where

$$A = B - Y_i P^i. \quad (29)$$

We have further

$$X_i = (X_i, Z)$$

$$n^i = n^{r+1} (-P^i, 1). \quad (30)$$

It is interesting to note that in going from the homogeneous problem to the inhomogeneous problem, it is always possible to eliminate n^{r+1} .

(b) If we have given the general variation principle

$$E = \int A \left(X_i, T, \frac{dX_i}{dT} \right) dT, \quad (31)$$

we introduce the vectors

$$G^i = \frac{\partial A}{\partial X_i} \quad (32)$$

$$N^i = \frac{\partial A}{\partial X_i'}, \quad X_i' = \frac{dX_i}{dT}.$$

Let us now introduce

$$\begin{aligned} X_i &= x_i(s) \\ T &= x_{r+1}(s). \end{aligned} \quad (33)$$

We then obtain

$$E = \int A \left(x_i, x_{r+1}, \frac{\dot{x}_i}{\dot{x}_{r+1}} \right) \dot{x}_{r+1} ds = \int a(x_i, \dot{x}_i) ds \quad (34)$$

where a is homogeneous in the first order of x_i .

We find that

$$n^i = \frac{\partial a}{\partial x_i} = (N^i, B) \quad (35)$$

with

$$B = A - N^i X'_i. \quad (36)$$

V. *The General System of Transversal Curves.*—If $a \neq 0$, $b \neq 0$, we have a variation principle, as well as a partial differential equation, determining our curves. We have in both cases an intermediate state between (III) and (IV). The variation problem does not depend on T . It is conservative and the partial differential equation does not depend on the dependent variable, Z . In both cases we are able to interpret our problem in the n -dimensional space.

However, our n^i no longer determines a contact transformation but only a canonical transformation, and our vector n^i is not orthogonal to the solution surfaces of the partial differential equation. We can write

$$b \left(x_i, \frac{\partial \phi}{\partial x_i} \right) = 0 \quad (37)$$

with

$$\phi(x_i) = z, \quad dz = n^i dx_i$$

and obtain, besides equation (8), the equation

$$z = \int n^i dx_i = \int a ds. \quad (38)$$

This general transformation was used by Jacobi to transfer Hamilton's methods from the calculus of variations to the theory of partial differential equations.

VI. *Conclusions.*—It might be of interest to the physicist to note that the following problems are mathematically equivalent:

- (1) The existence of an "energy integral," that is, a function $b(x_i, n^i)$, which remains unchanged along our curves.
- (2) The existence of a conservative variation principle.

(3) The existence of a differential (integral) invariant, the "flux."

(4) The wave notion, that all curves emerging from a point have accompanying wave surfaces, such that the vector n' is orthogonal to the surfaces at every intersection point.

In physical terms (1) is the most plausible assumption. The fact proved here might explain why the variation principle plays such a rôle in modern and classical physics.

* Communication No. 668.

Bruns, E., *Das Eikonal*, Leipzig, S. Hirzel (1895).

Carathéodory, C., *Variationsrechnung und partielle Differential-gleichungen erster Ordnung*, Leipzig-Berlin, B. G. Teubner (1935).

Cartan, E., *Leçons sur les Invariants Intégraux*.

Hamilton, W. R., *Mathematical Papers*, 1, Cambridge University Press (1931).

Herzberger, M., *Strahlenoptik*, Berlin, J. Springer (1931).

Herzberger, M., *On the Fundamental Invariant, the Optical Tetrality Principle and on the New Development of Gaussian Optics Based on This Law*, *Jour. Opt. Soc. Amer.*, 25, 295-304 (1935).

Huyghens, Christian, *Dioptrica* (1653, 1666), *Oeuvres*, 13, Haag, M. Nyhoff (1916).

Jacobi, C. G. I., *Zur Theorie der Variationsrechnung und der partiellen Differential-gleichungen*, *Ges. Werke*, Bd. V, 41-55.

Lagrange, J. L., *Memoire sur la Théorie Générale de la Variations des Constantes Arbitraires dans tous les Problèmes de Mécanique*, *Oeuvres*, Tome VI, 809-816.

Lie, S., *Ges. Abhandlungen*, Bd. III and IV, Leipzig, B. G. Teubner (1922, 1929).

Poincaré, *Les Méthodes Nouvelles de la Mécanique Céleste*, Gauthiers-Villars (1892-1899).

Prange, G., *Die Hamilton-Jacobische Theorie für Doppelintegrale*, Diss. Göttingen, (1915).

Straubel, R., "Über einen allgemeinen Satz der Geometrischen Optik und Seine Anwendungen," *Phys. Zeit.*, 4 (1902-1903).

THE RELATIVE NUMBERS OF THE SUBGROUPS AND OPERATORS OF CERTAIN GROUPS

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated August 19, 1938

Various groups contain more subgroups than operators while others contain more operators than subgroups. The group of lowest order which contains at least ten times as many subgroups as operators is the abelian group of order 32 and of type 1^4 , and the group of lowest order which contains at least ten times as many operators as subgroups has for its order the number obtained by reversing the digits of the order of the preceding group, if we include among the subgroups of a group the identity and the

group itself, as will be done in the present article. It is easy to see that there is no upper limit to the possible ratio of the number of the subgroups to the number of the operators of a given group since the number of the subgroups of order 4 in the abelian group of order 2^m and of type 1^m is $(2^m - 1)(2^m - 2)/6$, $m > 1$. Similarly, there is no upper limit to the possible ratio of the number of the operators of a given group to the number of its subgroups since the group of order p , p being a prime number, has only two subgroups.

Since the order of every subgroup is a divisor of the order of the group the number of the subgroups of a group of a given finite order is necessarily finite and cannot exceed the sum of the numbers of the combinations of its operators in sets equal to the divisors of its order. The simple group of smallest composite order has one more operator than subgroups since the number of the latter is 59. On the contrary, the simple group which has next to the smallest such order has slightly more subgroups than operators since it has 21 subgroups of order 2, 28 of order 3, 35 of order 4, 28 of order 6, 8 of order 7, 21 of order 8, 14 of order 12, 8 of order 21, 14 of order 24, besides the identity and the group itself, making a total of 179 subgroups, which is 11 more than the number of its operators. Little has been done toward the development of methods for determining the number of the subgroups of a given group.

In the case of Sylow subgroups it is comparatively easy to determine the number of the subgroups in view of the fact that all of these subgroups of the same order are conjugate under the group. The number of the Sylow subgroups of a given order in a group is obviously equal to the number of the subgroups of the group which are separately composed of all the operators of the group which transform such a Sylow subgroup into itself. This explains why in the simple group of order 168 there are as many subgroups of order 21 as there are subgroups of order 7, and as many subgroups of order 6 as there are subgroups of order 3. It may be noted that when this particular group is represented as an intransitive group of degree 15 it contains no substitution of this degree in view of the fact that its subgroups of orders 21 and 24 involve at least one substitution from each of its sets of conjugate substitutions of every order contained in the group.

When G is the cyclic group of order $p_1^{m_1}, p_2^{m_2}, \dots, p_i^{m_i}, p_1, p_2, \dots, p_i$ being distinct prime numbers, then the number of the subgroups of G is $(m_1 + 1)(m_2 + 1), \dots, (m_i + 1)$, since it is equal to the number of the positive integral divisors of the order of G . When G is dihedral and of the given order one of the given prime numbers, say p_1 , is 2 and the number of the subgroups of G can then be determined by observing that G then contains a cyclic subgroup of order $p_1^{m_1-1}, p_2^{m_2}, \dots, p_i^{m_i}$ and each of its subgroups which is not contained therein has exactly half of its operators therein. The number of the latter subgroups which involve a particular

subgroup of this cyclic subgroup is equal to the index of this subgroup under this cyclic subgroup. In particular, when $l = 1$ the number of the subgroups contained in G is $m_1 + 2^{m_1-1} + 2^{m_1-2} \dots + 1 = m_1 + 2^{m_1} - 1$ as has been noted before.

To determine the number of subgroups of G when $l > 1$ it may be observed that G could be constructed by dimidiating the dihedral groups of orders $2^{m_1}, 2p_2^{m_2}, \dots, 2p_l^{m_l}$ with respect to their separate cyclic subgroups. The number of the subgroups in the direct product of these cyclic subgroups is $m_1(m_2 + 1) \dots (m_l + 1)$, $l > 1$, according to the preceding paragraph. The number of the subgroups in the dihedral group of order $2p_\alpha^{m_\alpha}$, $\alpha > 1$ is $m_\alpha + 1 + p_\alpha^{m_\alpha} + p_\alpha^{m_\alpha-1} + \dots + 1 = m_\alpha + 1 + (p_\alpha^{m_\alpha+1} - 1)/(p_\alpha - 1)$. As the number of these subgroups which do not appear in the given cyclic subgroup is the product of the numbers of the subgroups in these dihedral groups which do not appear in their respective cyclic subgroups the total number of subgroups in the given dihedral group is given by the following formula:

$$m_1(m_2 + 1) \dots (m_l + 1) + (2^{m_1} - 1) p_2^{m_2+1} - 1)/(p_2 - 1) \\ \dots (p_l^{m_l+1} - 1)/(p_l - 1).$$

Hence some dihedral groups contain more subgroups than operators while with respect to others the reverse is true. In particular, the number of the operators in the dihedral group of order $2p^m$, p being an odd prime number, exceeds the number of subgroups in this group except when the group is of order 6. In this special case these two numbers are equal to each other.

When G is the dicyclic group of order 2^m then $m > 1$ and the number of its subgroups is $m + 2^{m-2} + 2^{m-3} + \dots + 1 = m + 2^{m-1} - 1$. Hence the number of the operators of such a group always exceeds the number of its subgroups. When G is the dicyclic group of order $4p^m$, p being an odd prime number, it contains $2m + 2 + p^m + p^{m-1} + \dots + 1 = 2m + 2 + (p^{m+1} - 1)/(p - 1)$ subgroups. Hence when G is the dicyclic group of order $p_1^{m_1} p_2^{m_2} \dots p_l^{m_l}$, p_1, p_2, \dots, p_l being distinct prime numbers and $p_1 = 2$, $m_1 \geq 2$, the number of the subgroups of G is

$$m_1(m_2 + 1) \dots (m_l + 1), l > 1, + (2^{m_1-1} - 1) (p_2^{m_2+1} - 1)/(p_2 - 1) \\ \dots (p_l^{m_l+1} - 1)/(p_l - 1).$$

In the abelian group of order p^m and of type 1^m , m being fixed, the number of the subgroups exceeds the number of the operators for various values of m when p does not exceed some given number but the value of p can always be increased so that the number of the operators exceeds the number of the subgroups, since the exponent of p in the expressions which give the numbers of the subgroups is lower in the denominators than in the numerators of the fractions which appear in these expressions.

Since the number of the subgroups of an abelian group whose order is not a power of a prime number is equal to the product of the numbers of its subgroups contained in its Sylow subgroups it results from the preceding paragraph that in every abelian group of a given type it is possible to select a prime factor of its order so large that the number of operators in the abelian group exceeds the number of its subgroups. It therefore results that in an abelian group it is not possible to say that the number of the subgroups exceeds the number of the operators from the fact that the type alone is given. On the other hand it is sometimes possible to say that the number of the operators is at least equal to the number of the subgroups of a given group from the type alone. In particular, when the group is cyclic it contains at least as many operators as subgroups. When the group is abelian and of type 1,1,1 the number of the subgroups exceeds the number of the operators when $p < 5$, but when $p > 3$ the number of the operators exceeds the number of the subgroups.

To determine the number of the subgroups of a prime power abelian group it should be noted that a necessary and sufficient condition that an abelian group of order p^m and of type m_1, m_2, \dots, m_i contains a subgroup of a given type m_1', m_2', \dots, m_i' is that the m 's can be associated with m' 's so that each m is at least as large as the corresponding m' . The number of different types of subgroups can therefore be determined by first noting the different types of possible subgroups and then determining the number of the subgroups of each of these types. The number of the different types of subgroups of any abelian group is the product of the number of different types of subgroups in its Sylow subgroups.

To determine the former of these numbers we may assume that the m 's are so arranged that each of them is at least as large as any one of those which follow it. Then we may take all of these m 's except the last and replace the last successively by $m_i, m_i - 1, \dots, 0$. We then take all the m 's except the last two and replace m_{i-1} by $m_{i-1} - 1$ and if m_i does not exceed $m_{i-1} - 1$ we replace it by $m_i, m_i - 1, \dots, 0$. If m_i exceeds $m_{i-1} - 1$ we replace it by $m_i - 1, m_i - 2, \dots, 0$. We then start with $m_{i-1} - 1$ and proceed as before, observing that no number is followed by a larger number. In particular, when G is of type m_1, m_2 , the number of the possible types of subgroups is $(m_1 - m_2 + 1)(m_2 + 1) + m_2 + m_2 - 1 + \dots + 1$.

It should be observed that the number of the possible types of subgroups is independent of the value of the prime number p but the number of the possible subgroups generally depends upon this prime number. A necessary and sufficient condition that the number of the types of subgroups of an abelian group is equal to its total number of subgroups is that the group is cyclic.

ON INTERPOLATION AND APPROXIMATION BY FUNCTIONS ANALYTIC AND BOUNDED IN A GIVEN REGION

By J. L. WALSH

DEPARTMENT OF MATHEMATICS, HARVARD UNIVERSITY

Communicated August 20, 1938

The general problem of interpolation and approximation to an arbitrary given analytic function by means of polynomials or more general rational functions, is an important one on which the existing literature is large, including a recent book by the present writer.¹ Analogous to this problem is the new but related problem of interpolation and approximation to an arbitrary given analytic function by means of functions required to be analytic and to have a given bound in a given region. The new problem is perhaps more difficult than the old, but nevertheless some of the technique that has already been developed for the former problem applies with relatively minor modification to the study of the new problem. It is the object of the present note to indicate briefly some of these modifications and their consequences. The present note represents, however, only the beginnings of the study of the new problem, and many open questions are left untouched.

I.—PROBLEM I. *Let the closed point set S lie interior to the region R , and let the function $f(z)$ be analytic on S but let $f(z)$ (considered together with its possible analytic extensions) not be analytic throughout the interior of R . Given $M > 0$, to study the function or all functions $F_m(z)$ analytic and of modulus not greater than M in R , such that the quantity*

$$m_M = \max [|f(z) - f_M(z)|, z \text{ on } S] \quad (1)$$

is a minimum; thus $f_M(z)$ is a function of best approximation to $f(z)$ on S ; and to study the behavior of m_M and $f_M(z)$ as M becomes infinite.

There must exist at least one function $f_M(z)$ for which m_M as defined by (1) is a minimum. For let $M > 0$ be given, let m_M denote the greatest lower bound of all the quantities

$$\max [|f(z) - f_M(z)|, z \text{ on } S],$$

where $f_M(z)$ is analytic and of modulus not greater than M in R , and let us suppose $\lim_{M \rightarrow \infty} m_M^{(k)} = m_M$,

$$m_M^{(k)} = \max [|f(z) - f_M^{(k)}(z)|, z \text{ on } S], \quad (2)$$

where $f_M^{(k)}(z)$ is analytic and of modulus not greater than M in R . The functions $f_M^{(k)}(z)$ form a normal family in R , and some subsequence $f_M^{(k)}(z)$ (new notation), converges uniformly in every closed subregion of R to some

limit function $f_M^{(o)}(z)$ which is analytic and of modulus not greater than M in R . But we have for z on S

$$\begin{aligned} \max |f(z) - f_M^{(o)}(z)| &\leq \max |f(z) - f_M^{(k)}(z)| \\ &+ \max |f_M^{(k)}(z) - f_M^{(o)}(z)| \\ &\leq m_M^{(k)} + \max |f_M^{(k)}(z) - f_M^{(o)}(z)|, \end{aligned}$$

which approaches m_M as k becomes infinite. Thus $f_M^{(o)}(z)$ satisfies the requirements of minimizing (1).

The question of the uniqueness of the minimizing function $f_M(z)$ is still open.

Under the conditions laid down, if S has a limit point interior to R , we must have $m_M > 0$, for $f(z)$ and $f_M(z)$ could not coincide on S without coinciding throughout R . Under these conditions the number m_M obviously decreases monotonically or remains unchanged as M increases.

Our fundamental theorem in connection with Problem I is

THEOREM 1. *Let R be a region with boundary B consisting of a finite number of components none of which is a single point. Let S be a closed set interior to R , with boundary C consisting of a finite number of components none of which is a single point, and let $R - S$ be a connected set. Let $U(x, y)$ denote the function which is harmonic in $R - S$, continuous in the corresponding closed region, which has the value zero on B and unity on C . Let C_ρ denote generically the locus $U(x, y) = \rho$, $0 < \rho < 1$ interior to $R - S$. Let R_ρ , $0 < \rho < 1$, denote generically the point set composed of S plus the points of $R - S$ at which we have $\rho < U(x, y) < 1$; then R_ρ consists of a finite number of subregions of R bounded by the entire locus C_ρ .*

Let the function $f(z)$ be analytic on S , but let there exist no function analytic throughout the interior of R which coincides with $f(z)$ on S . Then for the functions $f_M(z)$ of best approximation defined in the formal statement of Problem I we have

$$\lim_{M \rightarrow \infty} f_M(z) = f(z) \tag{3}$$

uniformly on S .

Indeed, if $f(z)$ is analytic throughout R_ρ , $0 < \rho < 1$, but does not coincide with any function analytic throughout a set $R_{\rho'}$, $0 < \rho' < \rho$, then (3) is valid throughout R_ρ , uniformly on any closed set interior to R_ρ , and we have

$$\lim_{M \rightarrow \infty} [\max |f(z) - f_M(z)|, z \text{ in } \bar{R}_\sigma]^{1/\log M} = e^{(\rho - \sigma)/\rho}$$

where $\sigma > \rho$ is arbitrary, and where $\bar{R}_\sigma = R_\sigma + C_\sigma$.

The set S need not be connected, and if S is not connected the monogenic function $f(z)$ defined on one component of S need have no relation whatever to the function $f(z)$ defined on the other components. But $f(z)$ is

analytic on S , hence can be extended analytically so as to be analytic throughout some point set R_ρ . We denote by ρ the smallest of all numbers σ such that $f(z)$ can be extended analytically from S so as to be single-valued and analytic throughout the point set R_σ ; the smallest σ , namely ρ , must exist (cf. op. cit. p. 58). Either a singularity of $f(z)$ lies on C_ρ ; or C_ρ has at least one multiple point A at which the various analytic extensions of $f(z)$ from different components of S along paths in R_ρ do not coincide (in the sense that the various functions fail to be identical throughout a neighborhood of A); or both of these eventualities occur.

It is no loss of generality to assume, as we shall do, that R is finite and bounded by a finite number of analytic Jordan curves. For if the components of the complement of R are denoted by B_1, B_2, \dots, B_l , we may map successively on the interior of a circle the complement of B_1 , the complement of the transform of B_2 , the complement of the last transform of B_3 and so on. Then R is mapped onto a finite region bounded by a finite number of analytic Jordan curves. It is to be noticed that the entire content of Theorem 1 is invariant under an arbitrary one-to-one conformal map.

The function $U(x, y)$, harmonic and of constant value zero on the l analytic Jordan curves comprising B , can be extended harmonically across B . Let us choose $\eta > 0$ so small that the locus $B' : U(x, y) = -\eta$ consists of l analytic Jordan curves exterior to R in the respective neighborhoods of the l curves comprising B . Choose η also so small that the locus $C_{1-\eta}$ (henceforth denoted by C') has no multiple points. Denote by S' the closed set $R_{1-\eta} + C_{1-\eta}$. Denote by R' the point set S plus the points at which we have $-\eta < U(x, y) < 1$, so that R' is bounded by B' and has $R + B$ as a closed subregion. Introduce the notation

$$U'(x, y) = U(x, y) + \eta, \quad (4)$$

so that $U'(x, y)$ is zero and unity on B' and C' , respectively, and is harmonic in the closed region whose interior points are $R' - S'$, bounded by B' and C' . Let C'_ρ denote generically the locus $U'(x, y) = \rho$, $0 < \rho < 1$, interior to $R' - S'$, let R'_ρ denote the set S' plus the points of $R' - S'$ at which we have $\rho < U'(x, y) < 1$, and let $\bar{R}'_\rho = R'_\rho + C'_\rho$.

We shall make essential use of the interpolation series of rational functions introduced by the writer² (op. cit., § 8.7) whose poles α_n (depending only on n) are uniformly distributed on B' with respect to the parameter $-V'$, and whose points of interpolation β_n are uniformly distributed on C' with respect to the parameter $+V'$, where V' is a function conjugate to U' in the region $R' - S'$. An arbitrary function $f(z)$ analytic throughout R'_ν , $0 < \nu < 1$, but not analytic throughout any $R'_{\nu'}$, $0 < \nu' < \nu$, can be expanded in a series of interpolation of the form

$$f(z) = a_0 + a_1 \frac{z - \beta_1}{z - \alpha_1} + a_2 \frac{(z - \beta_1)(z - \beta_2)}{(z - \alpha_1)(z - \alpha_2)} + \dots, \quad (5)$$

where we have the relations³

$$\lim_{n \rightarrow \infty} \left| \frac{(z - \beta_1) \dots (z - \beta_n)}{(z - \alpha_1) \dots (z - \alpha_n)} \right|^{1/n} = \exp \left[\frac{-2\pi}{\tau} U'(x, y) \right], \quad (6)$$

$$\overline{\lim}_{n \rightarrow \infty} |a_n|^{1/n} = e^{2\pi\tau/\tau} \quad (7)$$

and for $\mu > \nu$

$$\overline{\lim}_{n \rightarrow \infty} \left[\max |f(z) - r_n(z)|, z \text{ on } \bar{R}'_\mu \right]^{1/n} = e^{-2\pi(\mu-\nu)/\tau}, \quad (8)$$

where $r_n(z)$ is the sum of the first $n + 1$ terms of the right-hand member of (5), and where τ is a positive number defined by the equation $\tau = -\int_B dV'$. Then τ is independent of η .

We are now in a position to establish Theorem 1, identifying the given $f(z)$ with $f(z)$ in (5). In particular we have from (4) the equation $\nu = \rho + \underline{\eta}$. In equation (8) the left-hand member is not increased if we replace \bar{R}'_μ by C' , and then we may allow μ to approach unity:

$$\overline{\lim}_{n \rightarrow \infty} [\max |f(z) - r_n(z)|, z \text{ on } C']^{1/n} \leq e^{-2\pi(1-\rho-\eta)/\tau}. \quad (9)$$

By equations (6) and (7) we have ($0 < \lambda < \nu$)

$$\overline{\lim}_{n \rightarrow \infty} [\max |r_n(z)|, z \text{ on } C'_\lambda]^{1/n} \leq e^{-2\pi(\lambda-\rho-\eta)/\tau}. \quad (10)$$

Let $\epsilon > 0$ be arbitrary. A consequence of (9) is for z on S

$$|f(z) - r_n(z)| \leq M_1 e^{-2\pi n(1-\rho-\eta-\epsilon)/\tau}, \quad (11)$$

where M_1 is suitably chosen, and a consequence of (10) is for z on B [$U'(x, y) = \eta$ on B]

$$|r_n(z)| \leq M_2 e^{-2\pi n(-\rho-\epsilon)/\tau}, \quad (12)$$

where M_2 is suitably chosen.

When M is given greater than or equal to the right-hand member of (12), for $n = 1, 2, \dots$, we have actually exhibited a function $r_n(z)$ analytic and of modulus not greater than M in R such that inequality (11) is satisfied on S . Surely the function of best approximation $f_n(z)$ approximates to $f(z)$ on S as well as does the function $r_n(z)$:

$$|f(z) - f_n(z)| \leq M_1 e^{-2\pi n(1-\rho-\eta-\epsilon)/\tau}. \quad (13)$$

By virtue of (11) and (13) we may write for z on S

$$|r_n(z) - f_n(z)| \leq 2M_1 e^{-2\pi n(1-\rho-\eta-\epsilon)/\tau}. \quad (14)$$

The number $M > 0$ is now supposed given, and becomes infinite. We choose n the largest integer so that the right-hand member of (12) is less than or equal to M . By the relation $|f_M(z)| \leq M$ in R and by (12) we have for z on B

$$|r_n(z) - f_M(z)| \leq 2M_2 e^{-2\pi(n+1)(-\rho-\epsilon)/r}. \quad (15)$$

To be sure, $f_M(z)$ is conceivably not originally defined on B itself, but (15) is true in the sense of (Fatou) limiting values as z approaches B .

In the region $R - S$ we have the harmonic function $U(x, y)$ and have two inequalities (14) and (15) for the function $r_n(z) - f_M(z)$ on two loci $U(x, y) = \text{const.}$ By an extension of Hadamard's Three-Circle Theorem, a consequence of the Zweikonstantensatz,⁴ we have from (14) and (15) for z on C_σ

$$|r_n(z) - f_M(z)| \leq [2M_1 e^{-2\pi n(1-\rho-\eta-\epsilon)/r}]^\sigma \cdot [2M_2 e^{-2\pi(n+1)(-\rho-\epsilon)/r}]^{1-\sigma}, \quad (16)$$

$$\overline{\lim}_{n \rightarrow \infty} [\max |r_n(z) - f_M(z)|, z \text{ in } \overline{R}_\sigma]^{1/n} \leq e^{-2\pi(\sigma-\sigma\eta-\rho-\epsilon)/r}. \quad (17)$$

When $\sigma > \rho$ is given, $0 < \sigma < 1$, it is possible to choose ϵ and η so small that the exponent in the right-hand member of (17) is negative. By equation (8) it now follows that the set of functions $f_M(z)$ converges to $f(z)$ uniformly on \overline{R}_σ , $\sigma > \rho$. Indeed, from (8) and (17) we have

$$\overline{\lim}_{n \rightarrow \infty} [\max |f(z) - f_M(z)|, z \text{ in } \overline{R}_\sigma]^{1/n} \leq e^{-2\pi(\sigma-\eta-\rho-\epsilon)/r}. \quad (18)$$

Manipulation of inequality (18), by virtue of

$$M < M_2 e^{-2\pi(n+1)(-\rho-\epsilon)/r},$$

and by virtue of the arbitrariness of ϵ and η , yields

$$\overline{\lim}_{M \rightarrow \infty} [\max |f(z) - f_M(z)|, z \text{ in } \overline{R}_\sigma]^{1/\log M} \leq e^{(\sigma-\rho)/r}. \quad (19)$$

We now proceed to show that the *inequality* sign in (19) leads to a contradiction. Suppose for some $\sigma > \rho$ and for some $\epsilon > 0$ the inequality

$$[\max |f(z) - f_M(z)|, z \text{ in } \overline{R}_\sigma]^{1/\log M} \leq e^{(\sigma-\rho-\epsilon)/r} \quad (20)$$

holds for all M sufficiently large. Choose

$$M = e^{2\pi n \rho / r}, \quad n = 1, 2, 3, \dots, \quad (21)$$

and use the notation $F_n(z) = f_M(z)$, where M and n are related by (21). As in the proof of (16) [i.e., by the results of Nevanlinna] we have for z on C_σ , $\sigma > \rho > 0$,

$$|F_{n+1}(z) - F_n(z)| \leq [2e^{2\pi(n+1)\rho/\tau}]^{(-\nu+\sigma)/\sigma} \cdot [2e^{2\pi n(\rho-\sigma-\epsilon)/\tau}]^{\nu/\sigma}.$$

This right-hand member is, except for a factor independent of n ,

$$e^{2\pi n(\rho\sigma - \sigma\nu - \epsilon\nu)/\sigma\tau},$$

which approaches zero with $1/n$ provided merely $\nu > \rho\sigma/(\sigma + \epsilon)$, a number less than ρ . That is to say, inequality (20) implies the uniform convergence of the sequence $F_n(z)$ on every closed set interior to $R_{\rho\sigma/(\sigma+\epsilon)}$; the limit of the sequence $F_n(z)$ is $f(z)$ on S , hence is an analytic extension of $f(z)$ single-valued and analytic throughout the interior of $R_{\rho\sigma/(\sigma+\epsilon)}$, contrary to hypothesis. Theorem 1 is established.

We may obviously write

$$m_M = [\max|f(z) - f_M(z)|, z \text{ on } S] \leq [\max|f(z) - f_M(z)|, z \text{ in } \bar{R}_\sigma],$$

whence from (19)

$$\overline{\lim}_{M \rightarrow \infty} [\max|f(z) - f_M(z)|, z \text{ on } S]^{1/\log M} \leq e^{(\rho-1)/\rho}.$$

The impossibility of the inequality sign here follows precisely as did the impossibility of (20), so we have the

COROLLARY. Under the conditions of Theorem 1 we have also

$$\overline{\lim}_{M \rightarrow \infty} [\max|f(z) - f_M(z)|, z \text{ on } S]^{1/\log M} = e^{(\rho-1)/\rho}.$$

The additional result ($\sigma < \rho$)

$$\overline{\lim}_{M \rightarrow \infty} [\max|f_M(z)|, z \text{ in } \bar{R}_\sigma]^{1/\log M} = e^{(\rho-\sigma)/\rho}$$

may be established with the sign $=$ replaced by \leq from the definition of M and from the boundedness of $f_M(z)$ on C_μ , $\mu > \rho$, by the method of proof of (16). Impossibility of the sign $<$ follows as in the treatment of (19). By a similar method we obtain also

$$\overline{\lim}_{M \rightarrow \infty} [\max|f_M(z)|, z \text{ in } \bar{R}_\rho]^{1/\log M} = 1.$$

A limiting case of Problem I with for instance R the entire (finite) plane, S closed and limited, but consisting of an infinite number of points, and $f(z)$ analytic at every point of S but not at every point of R , is found by taking as norm of the approximating function $f_\alpha(z)$ not a bound on the modulus of $f_\alpha(z)$ but the number α , greatest lower bound of all numbers β for which

$$\lim_{z \rightarrow \infty} \frac{f_\alpha(z)}{z^\beta} = 0.$$

There are only a countable set of functions $f_\alpha(z)$ of best approximation, namely, the set of polynomials of best approximation to $f(z)$ on S . The analog of Theorem 1 is valid under suitable restrictions on S (op. cit., §5.1).

II.—PROBLEM II. *Let the closed point set S lie interior to the region R , and let the function $f(z)$ be analytic on S but let $f(z)$ (considered together with its possible analytic extensions) not be analytic throughout the interior of R . Given $m > 0$, to study the function (or all functions) $f_m(z)$ analytic in R such that*

$$|f(z) - f_m(z)| \leq m, \quad z \text{ on } S, \quad (22)$$

and such that the least upper bound

$$M_m = \text{l.u.b. } [|f_m(z)|, z \text{ in } R] \quad (23)$$

is least; and to study the behavior of M_m and $f_m(z)$ as m approaches zero.

Under the conditions of Problem II there may for given m exist no function $f_m(z)$ analytic in R satisfying (22) and (23); for instance, $R: |z| < 2$, $S: |z| = 1$, $f(z) = 1/z$; compare op. cit., p. 343. But if for a given m there exists a function $f_m(z)$ for which (22) is satisfied and for which $\text{l.u.b. } [|f_m(z)|, z \text{ in } R]$ is finite, there exists at least one function $f_m(z)$ satisfying (22) for which M_m as defined by (23) is least; compare the corresponding proof given in I.

Precisely the method of proof of Theorem 1 yields

THEOREM 2. *Let the region R , the point set S , and the function $f(z)$ satisfy the conditions of Theorem 1. With the notation of that theorem, we have for the functions $f_m(z)$ defined in the formal statement of Problem II*

$$\lim_{m \rightarrow 0} f_m(z) = f(z) \quad (24)$$

uniformly on S .

Indeed, if $f(z)$ is analytic throughout R_ρ , $0 < \rho < 1$, but not throughout any $R_{\rho'}$, $0 < \rho' < \rho$, then (24) is valid throughout R_ρ , uniformly on any closed set interior to R_ρ , and we have

$$\overline{\lim}_{m \rightarrow 0} [\max |f(z) - f_m(z)|, z \text{ in } \bar{R}_\sigma]^{-1/\log m} = e^{(\rho - \sigma)/(1 - \rho)}, \quad (25)$$

where $\sigma > \rho$ is arbitrary and where $\bar{R}_\sigma = R_\sigma + C_\sigma$. We also have

$$\overline{\lim}_{m \rightarrow 0} [\text{l.u.b. } |f_m(z)|, z \text{ in } R]^{-1/\log m} = e^{\rho/(1 - \rho)},$$

and for $\sigma \leq \rho$,

$$\overline{\lim}_{m \rightarrow 0} [\max |f_m(z)|, z \text{ in } \bar{R}_\sigma]^{-1/\log m} = e^{(\rho - \sigma)/(1 - \rho)}.$$

III.—PROBLEM III. Let the points $\beta_{n1}, \beta_{n2}, \dots, \beta_{nn}$ not necessarily distinct lie interior to the region R . Let the function $f(z)$ be analytic in each point β_{nh} . Let $f_n(z)$ be the (or a) function which coincides with $f(z)$ in the points $\beta_{n1}, \beta_{n2}, \dots, \beta_{nn}$, which is analytic in R , and the least upper bound M_n of whose modulus in R is a minimum. To study the functions $f_n(z)$, especially the approach to $f(z)$ of the sequence $f_n(z)$, and to study the sequence M_n as n becomes infinite.

If the boundary of R contains at least one continuum B_1 not a single point, there exists at least one function which is analytic and bounded in R and coincides with $f(z)$ in the points $\beta_{n1}, \beta_{n2}, \dots, \beta_{nn}$. For the region R can be mapped onto a bounded region, and we may interpolate to $f(z)$ in the prescribed points by a polynomial. Consequently there exists at least one function $f_n(z)$; compare the proof given in I. If R is simply connected, the function $f_n(z)$ is known to be unique.⁶

The method of proof used in Theorem 1 applies also here, without great modification, and yields analogous results provided the points are uniformly distributed (op. cit., §§7.5, 8.7) on C or satisfy similar conditions. We state without proof only a relatively simple limiting case of the corresponding analog of Theorem 1:

THEOREM 3. Let R be the point set $|z| < 1$. Let the function $f(z)$ be analytic throughout the region $|z| < \rho < 1$, but not analytic through any region $|z| < \rho'$ with $\rho' > \rho$. Let us choose $\beta_{nh} = 0$, and let $f_n(z)$ denote the function defined in the statement of Problem III. Then we have

$$\lim_{n \rightarrow \infty} f_n(z) = f(z)$$

for $|z| < \rho$, uniformly for $|z| \leq \rho_1 < \rho$. Moreover we have

$$\overline{\lim}_{n \rightarrow \infty} [\max |f(z) - f_n(z)|, \text{ for } |z| \leq \rho_1 < \rho]^{1/n} = \rho_1/\rho,$$

$$\overline{\lim}_{n \rightarrow \infty} [\text{l.u.b. } |f_n(z)|, \text{ for } |z| < 1]^{1/n} = 1/\rho,$$

$$\overline{\lim}_{n \rightarrow \infty} [\max |f_n(z)|, \text{ for } |z| = \rho_2, \rho \leq \rho_2 < 1]^{1/n} = \rho_2/\rho.$$

IV.—The method of proof of Theorem 1, as of various similar theorems (op. cit.) concerning sequences of functions of best approximation may be characterized as (i) the exhibiting of an explicit sequence whose properties are known and which converges (at least roughly) as well as the sequence of best approximation, and (ii) use of this comparison sequence and its convergence properties to obtain the convergence properties of the sequence of functions of best approximation. In (ii), the present most convenient tool is the result of Nevanlinna, a generalization of Hadamard's Three-Circle Theorem. In previous work (op. cit.), the most useful tool is

S. Bernstein's Lemma, in suitably general form (op. cit., §4.6), which may be interpreted as a limiting form of the Three-Circle Theorem.⁶ Another convenient tool, which is of use especially in the study of Problem III, is Schwarz's Lemma, also a limiting form of the Three-Circle Theorem, as Julia has remarked.⁷

The methods just mentioned can be used in the further study of Problems I, II and III, for instance in investigating the following topics: (a) the use of expressions other than (1), such as line or surface integrals with possible norm functions, as the measure of approximation of a function to $f(z)$ on S ; (b) the use of a line or surface integral with a norm function as a bound for the approximating function, instead of least upper bound of modulus in R ; (c) study of exact regions of uniform convergence of approximating sequences (as in op. cit., § 4.8); (d) study of convergence and degree of convergence on the boundaries of those regions; (e) study of overconvergence in the sense of Ostrowski; (f) relinquishing the requirement that $R - S$ be connected; and whether or not S separates points of R from B allowing auxiliary conditions of interpolation for the approximating functions (as in op. cit., Chapter XI); (g) synthesis of Problems II and III (cf. op. cit., § 7.9); (h) study of the case that $f(z)$ is analytic but not bounded in R (the present methods apply without change); (i) lightening the restrictions on R and S in Theorems 1 and 2; (j) interpolation and approximation to given harmonic functions by functions harmonic and bounded in a region. The writer hopes to return to these questions.

¹ Interpolation and Approximation by Rational Functions in the Complex Domain, New York, 1935. References in the present note are to that book unless otherwise indicated. Terminology is uniform with that work.

² These functions seem to be the only rational functions ever exhibited which "belong to" a more or less arbitrary multiply connected region. Corresponding rational functions (in this case polynomials) had previously been introduced belonging to an arbitrary simply connected region bounded by a Jordan curve by Fejér, based on methods due to Hilbert.

In the present application it is only incidental that the variable locus B' is chosen as a locus $U(x, y) = \text{const.}$ It is entirely satisfactory, by a slight modification of the formulas involved, to choose B' as a more general variable boundary exterior to R but approaching B . This remark is of importance in the extension of Theorem 1 to more general regions R .

³ Op. cit., p. 211, the present equation (8) appears as an *inequality*, but for the present case that the $\alpha_{n,k}$ and $\beta_{n,k}$ are independent of n this inequality becomes an equality; see op. cit., §3.4, Theorem 5; the inequality \leq is sufficiently strong for the present purposes. The analog of (8) holds also, where R_μ is replaced by S' and μ by unity.

⁴ See R. Nevanlinna, *Eindeutige Analytische Funktionen* (Berlin, 1936), p. 42. Nevanlinna assumes the region in question bounded by a finite number of Jordan arcs, a situation that can be reached in the present case by the method we have used in the transformation of B .

⁵ Kakeya, *Science Reports of Tôhoku University*, 1915, pp. 297-311; see also op. cit., §10.8.

⁶ With the notation of op. cit. p. 83, write $M_2 = Kr_2^\alpha$, and allow r_2 to become infinite. Op. cit., p. 83, inequality (19) takes the form $M \leq M_1(r/r_1)^\alpha$, which is essentially Bernstein's Lemma.

⁷ Principes géométriques d'analyse, première partie (Paris, 1930), p. 108.

A NEW FOUNDATION OF NON-EUCLIDEAN, AFFINE, REAL PROJECTIVE AND EUCLIDEAN GEOMETRY

BY KARL MENGER

DEPARTMENT OF MATHEMATICS, NOTRE DAME UNIVERSITY

Communicated August 29, 1938

The concepts of the non-Euclidean plane geometry can be defined in terms of the notions joining and intersecting of geometric entities as follows:¹ We call a system of three lines a *maximal triangle* if (1) no two of them intersect, (2) through any point of each of the three lines there exists exactly one line which intersects neither of the other two lines. We say that two lines L and M are *parallel* if there exists a line N such that L, M, N form a maximal triangle. We say the point Q lies *between two points* P and R if (1) P, Q, R are distinct collinear points, (2) any line through Q intersects at least one line of any maximal triangle two sides of which pass through P and R , respectively. The point Q is said to lie *between three points* if (1) these points are not collinear, (2) Q does not coincide with any of them nor lies between any two of them, (3) any line through Q contains at least one point between two of the three points. The points Q_1, Q_2, \dots are said to *converge toward* Q if for any three points the following condition holds: If Q lies between the three points, then almost all points Q_i (i.e., all except at most a finite number) lie between them. Two pairs of points P, Q and P', Q' on two parallel lines L and L' will be called *congruent* under the following condition: Let M and N be lines through P' and Q' , respectively, such that L, M, N are mutually distinct and parallel, and let M' and N' be two lines through P and Q , respectively, such that L', M', N' are mutually distinct and parallel; then the line joining the point of intersection of M and M' , with the point of intersection of N and N' is parallel to both L and L' . Two pairs of points P, Q and P'', Q'' on two non-parallel lines L and L'' are said to be congruent if there exists a pair of points P', Q' congruent with both pairs and contained in a line L' parallel to both L and L'' .

However, these definitions hold not only in the Bolyai-Lobatchefski geometry but in any plane geometry admitting a model in the interior of some convex curve if congruency is defined in the Cayley-Klein way by

means of the cross-ratio with respect to the boundary. Among all these geometries that of Bolyai-Lobatchefski can be characterized by means of Pascal's theorem: We call a system of six lines L_1, \dots, L_6 a *maximal hexagon* provided that L_i and L_j are parallel if $|i - j| \equiv 1 \pmod{4}$ and not parallel if $|i - j| \equiv 0 \pmod{2}$. We now can characterize Bolyai-Lobatchefski geometry by assuming: If the three pairs of opposite sides of a maximal hexagon intersect, then the three points of intersection are collinear.

By modifying the assumptions of Bolyai-Lobatchefski geometry which imply the convexity of the plane model we can develop other non-Euclidean geometries which admit plane models in domains that are not convex, even not simply connected or not connected. In these geometries the lines passing through a point P and not intersecting a line L may fill two or more angular sectors so that we can speak of four or more parallels to L through P . Assumptions such as these may on the other hand serve to characterize types of domains in the plane and in higher dimensional spaces.

The foundation of affine geometry on the joining and intersecting operations led so far² only to a restricted affine geometry (to the theorems not dealing with the between-relation). The whole affine geometry can be developed ultimately in terms of the two projective operations by completing the non-Euclidean space whose projective theory was sketched above, or, what is equivalent, by first developing the theory of an open part of the affine space and then introducing points of infinity corresponding to the boundary and ultra-infinite points corresponding to the exterior. We shall develop the theory of a *closed* part of the affine plane and then introduce ideal points of one type.

We start with the concepts of joining (primary) points and of intersecting (primary) lines, the concepts of points and lines themselves being derivable from the operations of joining and intersecting in the usual way² (point as that which has no proper part except the vacuum V , line as that which is not a proper part of anything except the universe U). A system of three lines for which there exists no line intersecting exactly one of the three lines of the system we call a *maximal triangle*. Two lines are called *coextremal* if together with some third line they form a maximal triangle. If P_1, P_2, P_3 are three distinct points of a line we say that P_2 lies *between* P_1 and P_3 if any line passing through P_2 intersects at least one of any two coextremal lines S_1 and S_3 passing through P_1 and P_3 , respectively.

We now introduce *ideal points* as certain pairs of non-intersecting lines. (Although these pairs could be characterized at this point, for brevity this will be deferred.) Two pairs of lines S_1, S_2 and S_3, S_4 are said to define *equal ideal points* if S_1, S_2, S_3 contain two triangles of points that are perspective from a line. The primary points and the ideal points together

will be called *secondary points*. Three secondary points Q_1, Q_2, Q_3 are said to be *secondarily collinear* if there exist three pairs of primary lines S_i^1, S_i^2 ($i = 1, 2, 3$) defining Q_i , respectively, such that S_i^1 and S_j^2 have a primary point P_{ij}^1 in common, and such that the triangles $P_{12}^1, P_{23}^1, P_{31}^1$ and $P_{12}^2, P_{23}^2, P_{31}^2$ are perspective from a primary point. If the law of Desargues is postulated for the joining and intersecting of primary elements, then the equality of ideal points is transitive, and three secondary points which are primary points are secondarily collinear if and only if they lie on a primary line. The secondary lines are thus extensions of the primary lines, the latter appearing as closed segments of the former if we define: The secondary point Q_2 lies *between* the secondary points Q_1 and Q_3 if the three points lie on a secondary line and if there exists a primary point P and a primary line containing three primary points P_1, P_2, P_3 such that P_1 lies on a secondary line with P and Q_i ($i = 1, 2, 3$), and where P_2 lies between P_1 and P_3 in the sense above defined for primary points.

By formulating the postulates concerning points and betweenness on which affine geometry is ordinarily based, for the secondary points and their betweenness defined above, we get a foundation of affine geometry ultimately in terms of the notions joining and intersecting of primary elements.

We call *secondary line* any pair of distinct secondary points, the secondary lines Q_1, Q_2 and Q_2, Q_3 being considered as *equal* if the secondary points Q_1, Q_2, Q_3 are secondarily collinear. Assuming the postulates of affine geometry for the secondary points and their betweenness we see that for any given secondary line there exists exactly one secondary line that has no secondary point in common with it and passes through a given secondary point outside the given line. Thus we see that a primary line defines an ideal point with any primary line passing through a point outside except one.

If instead we assume that any two distinct primary lines determine an ideal point and that the postulates of projective geometry hold for the secondary points and lines introduced as above, then we get a development of the projective geometry ultimately based on the notions joining and intersecting in which a between-relation is defined for the points of each line after the omission of one point. That is to say, we can develop the geometry of the real projective space including the theories of order and continuity in terms of the notions joining and intersecting.

The algebra of the restricted affine geometry replaces the introduction of an additional undefined concept ("parallel") by a postulate in terms of joining and intersecting ("For each line and each point outside in a plane there exists exactly one line passing through the point and not intersecting the line"). Though by its existential character this postulate differs from the other postulates of the algebra of the restricted affine geometry² it still is analogous to assumptions of the algebra of numbers (existence of nega-

tive and reciprocal numbers). More complicated but essentially of the same logical structure is the foundation of non-Euclidean geometry which replaces the ordinary introduction of additional undefined concepts ("congruent," "between," etc.) by existential postulates concerning joining and intersecting. In the foundation of the whole affine geometry developed above we had to add postulates concerning ideal elements, i.e., classes of primary elements.

We shall now show that one can develop the whole of Euclidean geometry in terms of the notions joining and intersecting by adjoining to these undefined concepts merely a few "constants" in the sense of methodology (viz., four points P_1, \dots, P_4) somewhat of the type of the constants V and U involved in algebra of geometry.

We start with the theory of a real projective plane from which we remove (1) a line with the exception of four distinct points, (2) a point outside of this line, (3) another line not passing through this point.³ The next step is to complete the reduced plane by introducing (1) ideal points corresponding to all points on the line through P_1, \dots, P_4 except the one which it has in common with the other removed line, (2) an ideal point P_0 corresponding to the removed point outside the two removed lines. In this way we get an affine plane containing the four collinear points P_1, \dots, P_4 and the point P_0 which is not collinear with them. By means of the four constant points P_1, \dots, P_4 we can define, in terms of joining and intersecting exclusively, an involution without fixed element in the pencil of lines through P_0 . Two lines may be called *perpendicular* if they are parallel to or identical with two lines through P_0 that correspond to each other under this involution. Congruency, as well known, can be defined in any affine space for pairs of points which are situated on the same or on parallel lines. In our affine space with perpendicularity we can define moreover: Two pairs of points O, A and O, B where O, A, B are not collinear, are *congruent* if the line $A + B$ is perpendicular to the line $B + A'$ where A' is the point collinear with O and A but distinct from A for which the pairs O, A' and O, A are congruent. In this way all concepts of Euclidean geometry are ultimately expressed in terms of the notions joining and intersecting.

We can develop each of the geometries considered above, for instance, from its ordinary postulates after substituting in them for all undefined concepts involved their expressions in terms of joining and intersecting as they have been defined in this paper. But one could also start with simpler direct assumptions concerning the two projective operations.⁴

³ The main idea of this projective foundation of non-Euclidean geometry was presented to the American Mathematical Society at the April meeting, 1938, in a paper under press in the *Bull. Amer. Math. Soc.* The complete system of definitions of the non-Euclidean concepts is submitted for publication in the *Paris Compt. Rend.*

⁴ The author has sketched this method in the *Jahresbericht deutsch. Mathem. Ver.*, 37,

309 (1928) and carried it out in collaboration with F. Alt and O. Schreiber in the *Ann. Math.*, 37, 465 (1936). Projective, affine and non-Euclidean geometries of all dimensions deal with two commutative and associative operations, joining and intersecting of elements, either of which admits an indifferent element (a vacuum V and a universe U which do not affect any element by being joined or intersected with it, respectively). The elements form thus what G. Birkhoff calls a lattice, in an extended theory which he developed within the last years and, as far as geometry is concerned, applied to the projective case. Moreover, the operations of all the geometries mentioned above satisfy (1) an absorption law on which definitions of part, point and hyperplane can be based; (2) the assumption that each element is join of a finite number of points, and intersection of a finite number of hyperplanes; (3) the assumption that for any element A , if P is a point there is no element which has the proper part A and is a proper part of $A + P$, and if H is a hyperplane whose intersection with A is not vacuous there is no element which is a proper part of A and contains the proper part $A.H$. From these assumptions which one might call those of a *geometric lattice* we get projective geometry by adding the assumption that also if the hyperplane H has the intersection V with A no element is a proper part of A and contains $A.H = V$ as a proper part or, what is equivalent, no elements (except points) are parallel to hyperplanes. We get a restricted affine geometry according to F. Alt by adding Euclid's parallel postulate. We get non-Euclidean geometry by adding the assumptions specified in the papers quoted in ¹.

² Except for some technical complications this theory would be similar to that of a projective plane from which two distinct lines are removed. The latter theory can be derived from the following assumptions: (1) Through each point P there passes exactly one line L_P such that through any point Q outside of L_P there exists exactly one line that does not intersect L_P . (2) If L is any line through P different from L_P then there exist through any point outside of L exactly two lines neither of which intersects L .

³ For the non-Euclidean between-relation Mr. F. P. Jenks succeeded in deriving its main properties (postulated in the ordinary development of non-Euclidean geometry) from simple assumptions in terms of the joining and intersection operations.

A JORDAN SPACE CURVE HAVING THE INFINITE AREA PROPERTY AT EACH OF ITS POINTS

BY JESSE DOUGLAS

BROOKLYN, N. Y.

Communicated July 29, 1938

By the property specified in the title is meant the following. Let P denote an arbitrary point of the Jordan space curve J , S any sphere about P as center and Σ any surface bounded by J . Then the area of the portion of Σ included within S is infinite.

The author has constructed both geometric and analytic examples of Jordan curves having the infinite area property at a single point.¹ I have also given analytically a curve J having this property at every point.² But the example now to be presented is more geometrical and vivid, its possession of the specified property more clear intuitively.

The importance of these curves in the Plateau problem is well known. For when prescribed as contour for a required minimal surface, they do not admit of direct treatment either by the area functional or by the functional $A(g)$ introduced by the author, since both of these become identically infinite. A simple limit process, however, enables us to pass from finite-area-bounding contours (e.g., polygons) to the general case.³

1. LEMMA. *Let the Jordan curve J link N times with the torus T , the area of whose circular meridian section is M . Then the area of the portion intercepted by T of any two-sided surface Σ bounded by J is at least NM .⁴*

The simple proof of this lemma, based on a standard linkage theorem,⁵ and on the fact that orthogonal projection diminishes area, has been given in a previous note of the author's,⁶ where it was applied to the construction of a curve J_0 with the infinite area property at a single point.

The essential thought of the present paper is to combine the principle of construction of J_0 with a method of condensation of singularities.

2. *Chain of Spheres.*—We begin with the construction of a *closed chain* C_1 of spheres, each of which will be denoted by S_1 . The number m_1 of spheres in the chain is finite, each is externally tangent to its two neighbors, and otherwise no two spheres of the chain have either an interior or boundary point in common. The attribute *closed* implies a cyclic arrangement; i.e., the last sphere is externally tangent to the first. The picture of a full string of beads is appropriate.

Let the contact-points of any particular sphere $S_1^{(p)}$ with its two neighboring spheres be P', P'' . In the interior of $S_1^{(p)}$ let any torus $T_1^{(p)}$ be constructed; denote by M the area of its meridian section. Then construct a non-self-intersecting polygonal line Π leading from P' to P'' , starting and ending along a radius, contained entirely in the interior of $S_1^{(p)}$ (except for its end-points), and linking N times with $T_1^{(p)}$, where

$$N > \frac{1}{M}. \quad (1)$$

We can then easily form an *open chain* $C_2^{(p)}$ of spheres $S_2^{(p)}$ with centers on Π , each externally tangent to its two neighbors, otherwise mutually exclusive, the first and last internally tangent to $S_1^{(p)}$ at P', P'' , respectively; furthermore, we can choose the diameters of the spheres $S_2^{(p)}$ so small that the following conditions are satisfied:

- (i) each diameter $< 1/2^2$;
- (ii) each sphere $S_2^{(p)}$ is contained in the sphere $S_1^{(p)}$;
- (iii) the chain $C_2^{(p)}$ itself links N times with the torus $T_1^{(p)}$; i.e., no sphere $S_2^{(p)}$, with its center on Π , is large enough to reach to the torus.

If the preceding construction is carried out in each sphere $S_1^{(p)}$, then the sum of all the open chains so obtained:

$$C_2 = \sum_{p=1}^{m_1} C_1^{(p)} \quad (2)$$

is a *closed chain*, whose individual spheres will be denoted simply by S_2 (i.e., with omission of the index p indicating the particular sphere S_1 which contains S_2).

The chain of spheres C_1 is a first approximation, the chain C_2 a second approximation to the desired Jordan curve J .

The procedure whereby we have passed from C_1 to C_2 can be iterated indefinitely. We have only to make in the conditions (i), (ii), (iii) the following replacements: $1/2^2$ by $1/2^n$, index 2 throughout by index n , index 1 by $n - 1$. The linkage number N is always chosen in relation to the meridian sectional area M of each torus $T_n^{(p)}$ so that the condition (1) is obeyed.⁷ In this way we obtain for each integer n a closed chain C_n of spheres S_n . C_n is the n th approximation to our Jordan curve J ; in fact,

$$J = \lim_{n \rightarrow \infty} C_n. \quad (3)$$

Since, by construction, each chain C_n , considered as point-set, contains its successor C_{n+1} , we may also express J as the set-theoretic product of these chains:

$$J = C_1 \cdot C_2 \cdot \dots \cdot C_n \cdot \dots \quad (4)$$

3. *J is a Jordan Curve.*—The next step is to prove, as will easily be done, that J , as defined by the foregoing construction, is indeed a Jordan curve, i.e., continuous, closed and non-self-intersecting, or—what is equivalent—a topological image of a circumference.

Let K denote, then, any fixed circumference. Divide it into m_1 equal arcs A_1 — m_1 , we recall, is the number of spheres S_1 in the chain C_1 . Now make the arcs A_1 correspond, in order, to the spheres S_1 .

If $m_2^{(p)}$ is the number of spheres S_2 contained in any particular sphere $S_1^{(p)}$, let the corresponding arc $A_1^{(p)}$ be subdivided into $m_2^{(p)}$ equal parts A_2 , each of which is made to correspond to a sphere S_2 , in order; i.e., so that adjacent arcs A_2 correspond to adjacent, or externally tangent, spheres S_2 . Continuing in this way, we obtain a sequence of divisions Δ_n of the circumference K into arcs A_n , each Δ_n a subdivision of the preceding Δ_{n-1} , and where the length d_n of the longest arc⁸ of Δ_n tends to zero as n becomes infinite. (For $m_n^{(p)}$, the number of spheres S_n contained in any particular sphere $S_{n-1}^{(p)}$, is certainly greater than 2 in every case; therefore $d_n < d_{n-1}/2 < K/2^n$.)

Let p now denote any chosen point of K . Then p can always be represented as the unique common point of a "nest" of arcs: $\{A_n\} = \{A_1, A_2, \dots, A_n, \dots\}$, i.e., a sequence of arcs such that A_n contains A_{n+1} for every n , while the length of A_n tends to zero as $n \rightarrow \infty$. If p is not one of the

denumerable infinity of points marking any of the divisions Δ_n , the nest $\{A_n\}$ is evidently uniquely determined by p ; otherwise the determination of the nest by p is two-fold.⁹

Consider first the general case, where p is not a marking-point of any division Δ_n . Then the spheres S_n which correspond to the nest $\{A_n\}$ of arcs must clearly also form a nest $\{S_n\}$. For, by construction, the relation of inclusion among the arcs is paralleled by the same relation among the corresponding spheres; also, by condition (i) of the construction with n in place of 2, the diameter of any sphere S_n is less than $1/2^n$ and so tends to zero as n becomes infinite. The nest $\{S_n\}$ defines a unique point P belonging to all its spheres. We make this point P of space correspond to the point p of K .

If, on the other hand, p is a marking-point of a division Δ_n , then it determines two nests of arcs $\{A_n\}$.¹⁰ To these correspond two nests of spheres $\{S_n\}$, which evidently determine the same point P , for, clearly, every sphere of the one nest is externally tangent to every sphere of the other at the point P .

We now have a univocal transformation $p \rightarrow P$ proceeding from the points p of K to points P of space.

It is easily seen that the inverse of this transformation is also univocal; i.e., to different points p, q correspond different points P, Q . For if p, q are distinct, then, obviously, for a sufficiently large value of n they cannot belong to the same or to consecutive arcs A_n ;¹¹ therefore the corresponding points P, Q cannot belong to the same or to consecutive spheres S_n ; consequently, the respective spheres S_n to which they do belong are completely disjoint, for only consecutive spheres S_n can even touch one another; hence P and Q are distinct.

Finally, the transformation $p \rightarrow P$ is continuous. For let $\epsilon > 0$ be assigned arbitrarily. Choose n so large that $1/2^{n-1} < \epsilon$. Then any two points P, Q which belong to the same or to adjacent spheres S_n must have a distance apart $< \epsilon$, since the diameter of any sphere S_n is less than $1/2^n$. Let δ denote the minimum length¹² of any arc A_n of the division Δ_n . Then any two points p, q of K whose distance apart is less than δ must lie on the same or on adjacent arcs A_n ; hence their images P, Q lie in the same or in adjacent spheres S_n ; accordingly, the distance PQ is less than ϵ . Thus, precisely, the definition of continuity is fulfilled: "for every ϵ there is a δ , etc."

In sum, the correspondence of the points p of the circumference K to the points P of J is biunivocal and continuous; therefore, indeed, J is a Jordan curve.

4. *The Infinite Area Property of J.*—Let now Σ denote any two-sided surface bounded by J .

Since J passes in succession through the spheres S_{n+1} of the chain C_{n+1} ,

it follows from the preceding description that J links with each torus $T_n^{(p)}$ a number of times N related to the meridian sectional area M of $T_n^{(p)}$ according to the inequality (1).⁷ From the lemma of §1, it then follows that every torus $T_n^{(p)}$ intercepts on Σ at least one unit of area. Let S_n, S_{n+1} denote any spheres of consecutive chains such that S_n contains S_{n+1} ; then the shell between these two spheres contains a torus $T_n^{(p)}$; therefore every shell $S_n - S_{n+1}$ contains at least one unit of area of Σ . By repetition of this argument, it follows that every shell $S_n - S_{n+m}$ (where the latter sphere is included in the former) contains at least m units of area of Σ . But for any fixed particular sphere S_n , we can evidently choose S_{n+m} to belong to an arbitrarily large value of m . Therefore:

Any sphere S_n , occurring at any stage whatever in the construction of J , intercepts an infinite area on Σ .

Hardly any more need be said in order to arrive explicitly at the property of J specified at the beginning of this note. Only the observation is required that if P denote an arbitrary point of J and S any fixed sphere about P as center, then P is the limit point of a nest of spheres $\{S_n\}$; therefore, for a sufficiently large value of n , a sphere S_n is contained entirely within S .

The proof thus completed, like the lemma of §1 on which it is based, supposes the surface Σ to be two-sided. But, as explained in footnote 4, the result applies just as well to the case where Σ is one-sided.

¹ J. Douglas, "A Jordan Space Curve Which Bounds no Finite Simply-Connected Area," these PROCEEDINGS, 19, 269-271 (1933); "An Analytic Closed Space Curve Which Bounds no Orientable Surface of Finite Area," *Ibid.*, 19, 448-451 (1933).

² J. Douglas, "A Jordan Space Curve no Arc of Which Can Form Part of a Contour Which Bounds a Finite Area," *Ann. Math.*, 35, 100-103 (1934).

³ First carried out by the author, "Solution of the Problem of Plateau," *Trans. Amer. Math. Soc.*, 33, 263-321 (1931), particularly §§19, 20.

⁴ The precise meaning of "any surface Σ bounded by J " is: the locus of the end-point of a vector $\mathfrak{X}(u, v)$, continuous function of the point (u, v) which ranges over the interior and boundary of a riemannian manifold R of any topological structure whatever, \mathfrak{X} being required to convert the boundary B of R into J in a one-one continuous manner. The lemma does not apply if Σ is one-sided (see the first reference in footnote 1); nevertheless, the final result of this paper holds also for such surfaces. In fact, it follows from the author's formulas involving the fundamental functional $A(g, R)$ that the simply connected case is completely decisive as to the infinite area property: if every simply connected surface bounded by J has infinite area, the same will be true of every surface bounded by J , no matter what its topological type, including one-sidedness. Analytically, this is due to the fact that the "kernel" $P(z, \zeta)$ of the integral which expresses $A(g, R)$ has the same singular term $1/(z - \zeta)^2$ in all cases, regardless of the particular topological form of R . See the author's papers in *Journ. Math. Phys.*, 10, 328 (1931); 15, 105-123 (1936); also one now in press, which will appear in *Ann. Math.*, "Minimal Surfaces of Higher Topological Structure."

The area of a surface is to be understood in the sense of Lebesgue: as least limit of the areas of approaching polyhedra. To avoid whatever may be even slightly vague in

the notion of "area of the portion of Σ intercepted by T ," we may interpret the lemma as follows: "If the boundary of any two-sided polyhedral surface is sufficiently near to J (sense of Fréchet), then T intercepts from this polyhedral surface an area at least equal to NM ." Here only piece-wise plane areas bounded by algebraic curves intervene. Similarly, the final specific property of J may be interpreted as follows: "If the boundary of any polyhedral surface tends to J , then the area of that portion of the polyhedral surface which is contained within any fixed sphere S about any fixed point P of J as center tends to infinity."

⁶ If the continuous closed curve J' links N times with J , then every two-sided (orientable) surface bounded by J is intersected at least N times by J' . See G. Julia, *Exercices d'Analyse*, 1, 115–119 (1928).

⁶ The first citation in footnote 1.

⁷ Of course, M and N vary with the particular torus $T_n^{(p)}$.

⁸ Often called the *norm* of the division Δ_n .

⁹ In the standard logical foundation of the real number system, this corresponds to a unique representation of a non-terminating decimal as against the two different possible representations of a terminating one.

¹⁰ From a certain value of n on, one sequence of arcs A_n have their right end-points coincident with p , the other their left end-points. This is from the viewpoint of one located at p and facing along the radius.

¹¹ Let n be so large that d_n is less than half the arc length pq .

¹² To be clearly distinguished from d_n , the corresponding *maximum* length.

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 24

November 15, 1938

Number 11

Copyright 1938 by The National Academy of Sciences

*PARTIAL SELF-INCOMPATIBILITY IN MEDICAGO SATIVA**

BY R. A. BRINK AND D. C. COOPER

DEPARTMENT OF GENETICS, UNIVERSITY OF WISCONSIN

Communicated October 3, 1938

It has been known since the work of Piper, Evans, McKee and Morse¹ that cross-pollination of alfalfa increases the production of seed, as compared with close-pollination. Heretofore, the cause of this behavior has not been revealed. In the present paper it will be shown that the difference is associated with two phenomena, possibly unrelated, whose effects can be distinguished by a histological study of the pistil following the two types of matings. The plant is found to be partially self-incompatible (self-sterile); that is, male gametophytes, while not impotent on the individual from which they arise, are less effective in accomplishing fertilization than are unrelated male gametophytes. The second important reason for the difference in fertility, as measured by the number of functional seeds developed, is the markedly greater tendency for ovules containing inbred embryos rather than those resulting from cross-pollination to collapse during development.

The seven plants used in the experiment were the immediate offspring, by close-pollination, of six unrelated individuals selected from commercial varieties of alfalfa. They were grown in a greenhouse at 70–80°F., and the pollinations were made in April, 1938, at the height of the flowering period. Newly opened flowers on several inflorescences on each individual were castrated. Some of these were then close-pollinated by using pollen from other flowers on the same respective plants. The remaining castrated flowers were cross-pollinated, the pollen being derived in each case from a different individual within the group. Each of the plants, therefore, served as a pistillate and as a staminate parent in the crosses, in addition to being selfed. In making the matings, an abundance of pollen was brought into intimate contact with the stigmatic secretion by means of toothpicks tipped with a narrow strip of fine emery cloth. The pistils were collected at 30, 48, 72, 96, 120 and 144 hours after pollination, fixed and embedded in paraffin. After sectioning and staining, the follow-

ing data were taken on each pistil, giving regard to the serial order of the ovules from apex to base of the ovary: position of the most advanced pollen tubes, fertility of the ovules, fertile ovules collapsing and stage of development of the embryo. About nine pistils per plant were taken at each collection in the selfed series and about six pistils in the crossed group.

Few pollen tubes advance beyond the mid-region of the ovary after selfing, whereas, following cross-pollination, the tubes usually reach the base of the ovarian cavity. At the time of the first collection, 30 hours after pollination, the longest pollen tubes in the selfed series had reached nearly to the fourth ovule, on the average. At 48 hours the mean position lay between the fifth and sixth ovules. Pollen tubes were not found in the later collections, growth being completed, presumably, prior to 72 hours in both series. Following cross-pollination, on the other hand, the leading pollen tubes were found between the eighth and ninth ovules at 30 hours, and at the tenth ovule 18 hours later. In both series the frequency of ovules becoming fertile declines from apex to base of the ovary, particularly beyond the fourth or fifth ovule.

Counts made on ovaries taken at 72 hours, at which time pollen-tube growth has ceased, and at 96, 120 and 144 hours, show that the eggs in 66 per cent of the ovules were fertilized in the cross-pollinated series, as compared with only 14.5 per cent after selfing. All seven plants, while varying in degree of self-incompatibility, reacted alike in showing a large increase in percentage of fertile ovules on being cross-pollinated. The disparity is related in part, to pollen-tube length. That this factor, however, does not fully account for the difference is shown below.

Pollen tubes usually were found to have reached at least the first four ovules at the apical end of the ovary following both self-pollination and cross-pollination. Tabulation of the condition of the ovules in these positions at the intervals beyond 48 hours reveals the fact, however, that only 28 per cent became fertile after selfing, in contrast with 80 per cent after crossing. In numerous cases in the selfed series pollen tubes were seen which had passed directly by the micropyle of an ovule containing an unfertilized egg. This tendency of a pollen tube which has reached the micropyle of a normal ovule not to enter is a manifestation of self-incompatibility which seems not to have been established definitely for any other plant. Stout and Chandler² have shown that incompatibility reactions very probably occur in the ovary following certain matings in *Hemerocallis*. Little appears to be known in these cases, however, concerning the behavior of the pollen tubes after the ovarian cavity is entered.

Not all the alfalfa ovules in which development of an embryo and endosperm is initiated at the time of fertilization continue growth. It is of

interest that the mortality is far higher among those containing inbred embryos. In the collections made after 48 hours, 34 per cent of the fertile ovules in the selfed series were found to be collapsing, as compared with only 7 per cent following cross-pollination. Whether the higher mortality of fertile ovules after selfing is correlated with the self-incompatibility or is an inbreeding depression effect cannot be stated definitely from the present evidence. The results of a previous study³ of 10 plants in which no relationship was apparent between the proportion of ovules, becoming fertile after close-pollination and the frequency of abortion up to 120 hours suggest, however, that cessation of growth of the ovules may be an independent phenomenon.

Seed-formation in the alfalfa plant varies to an extraordinary degree, and the problem has yielded rather slowly to the attempts which have been made to elucidate it. It now appears that much of the difficulty which investigators have experienced in arranging well-directed experiments, and in interpreting the evidence from them, is due to the fact that more than one phase of the reproductive process is commonly subject to disturbance. Armstrong and White⁴ have called attention to the stigmatic membrane as a factor conditioning pollen-germination, and some additional observations by the present authors³ give further emphasis to this structure as an important variable. It is now shown that the alfalfa plant is partially self-incompatible. This fact also has far-reaching implications. A third, possibly independent, element which is significant for fertility is the collapse of fertile ovules during development. An effective approach to the general problem of seed-setting requires that attention be given to these distinct factors.

* Papers from the Department of Genetics, Agricultural Experiment Station, No. 234, and the Department of Botany, University of Wisconsin. The authors desire to express their appreciation for the support received from the Wisconsin Alumni Research Fund.

¹ Piper, C. V., Evans, M. W., McKee, R., and Morse, W. J., *U. S. Dept. Agr. Bull.*, 75 (1914).

² Stout, A. B., and Chandler, C., *Bull. Torrey Bot. Club*, 60, 397-416 (1933).

³ Brink, R. A., and Cooper, D. C., *Amer. Jour. Bot.*, 23, 678-683 (1936).

⁴ Armstrong, J. M., and White, W. J., *Jour. Agr. Sci.*, 25, 161-179 (1935).

MITOTIC BEHAVIOR OF INDUCED CHROMOSOMAL
FRAGMENTS LACKING SPINDLE ATTACHMENTS IN THE
NEUROBLASTS OF THE GRASSHOPPER

By J. GORDON CARLSON

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF ALABAMA, AND DEPARTMENT OF GENETICS,
CARNEGIE INSTITUTION OF WASHINGTON

Communicated October 14, 1938

The subject of the mitotic behavior and ultimate fate of x-ray induced fragments¹ has been almost entirely overlooked, or at least neglected, by most cytologists. Usually it is dismissed with the statement that fragments will not survive mitosis. This may be due in part to the use of material unsuitable for such studies, in part to the fact that fragments behave differently in different cells and in part to the interest of the investigator in other aspects of fragmentation. At most it has been noted that fragments lag at anaphase and are often included in small accessory nuclei at telophase.

The present study is based entirely on observations of the neuroblasts of the grasshopper, *Chortophaga viridifasciata*. Embryos were irradiated with 250 r, removed from the egg at such a time that cells treated in interphase or early prophase were in their first division, smeared on glass slips, fixed and stained in aceto-carmine, and mounted in euparal.

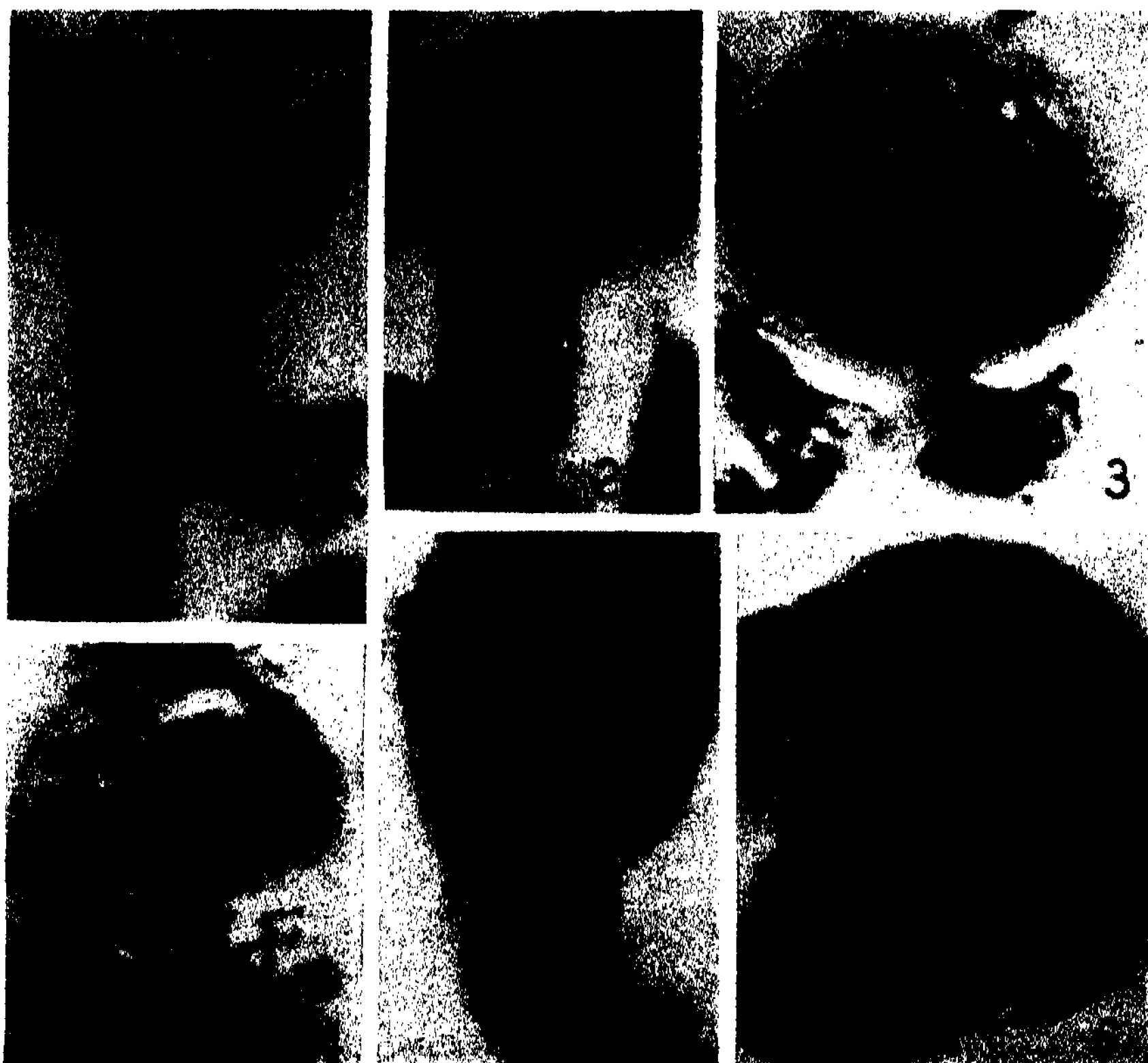
The results of this study throw light on certain factors involved in the mechanism of mitosis, because we are dealing here with chromosomal elements in which one complicating factor, namely, the spindle attachment region, is absent. Also, they indicate that the occurrence of delayed effects resulting from reattachments between chromosomes and fragments in a later mitotic cycle than that in which breakage occurred is not untenable cytologically.

Observations.—1. *Prophase.*—Fragments first become identifiable in the late prophase, when they differ from the chromosomes in that they lack the proximal heteropyknotic regions that mark the positions of spindle attachments. There is nothing in their behavior, their position in the nucleus, or their degree of condensation at this period to distinguish them from the chromosomes.

2. *Metaphase.*—The fragments lie between the cell membrane and the distal ends of the chromosomes. They are situated in the equatorial plane or at least as close to it as the distal ends of certain of the longest chromosomes, which often project slightly toward one or the other of the poles.

3. *Anaphase.*—A detailed analysis of the behavior of fragments at early anaphase appears in another paper.² The initial separation of their

"chromatids" begins at the same time as that of the chromosomes and gives rise to fragments of three classes: V's, rings and pairs of rods (Fig. 1). At first these are situated, as at metaphase, outside the distal ends of the



FIGURES 1-6

Fragments during and at the end of the first mitosis following their production. Dosage 250 r \times 990. 1—Anaphase. Two pairs of daughter fragments. Those in focus have not yet become oriented in relation to the spindle. Of the pair out of focus at left the lower member is oriented parallel to the spindle axis. 2—Anaphase. Three pairs of daughter fragments on the spindle moving toward the poles. The upper member of the large pair at left is much nearer the pole than any of the others. 3—Telophase. The darkly staining fragments are included in the cell nucleus. 4—Telophase. One or more fragments lying partly outside the cell nucleus appear joined to chromosomes inside it. 5—Telophase. Accessory nuclei in neuroblast and ganglion cell, probably containing sister fragments. 6—Interphase. Neuroblast with accessory nucleus containing dark staining fragments.

chromosomes and therefore a considerable distance from the spindle. At late anaphase, however, after the daughter chromosomes have become well

separated with an accompanying elongation of the cell, the fragments move inward between their distal ends, eventually coming in contact with the spindle. Once this occurs their "chromatids" gradually become oriented in a line with the spindle axis and begin to move toward opposite poles (Fig. 2). If the "chromatids" open out as a pair of rods, separation is complete from the first, and the daughter fragments can move unhindered toward the poles. If the fragment is V- or ring-shaped, with fusion at one or both ends of the fragment, respectively, this union may persist for a time, so that the fragment lags at the equator for a varying period.

4. *Telophase*.—The position of the cleavage plane that divides the cytosome of the original neuroblast into daughter neuroblast and daughter ganglion cell determines in which cell a given daughter fragment will be included. Unless a great many fragments with complex fusion patterns are present, sister fragments usually come to lie in different cells (Fig. 5). In one embryo studied 17 cells in late anaphase and early telophase contained 81 pairs of "chromatid" fragments. Sister "chromatids" of 51 of these pairs were situated in different daughter cells, sister "chromatids" of 8 lay in the same daughter cell, while the apportionment of the "chromatids" of 22 pairs could not be determined with any reasonable degree of certainty. Coincident with cytokinesis is the loss of stainability of chromosomes and fragments and the gradual formation of clear areas about them, the extent of which mark the limits of the future nucleus. This occurs in the fragments somewhat later than in the chromosomes. Fragments that are close to the distal ends of the chromosomes are included with them in the cell nucleus (Fig. 3). The conclusion of Mather³ in his studies of the post-meiotic resting stage of *Tradescantia*, *Eremurus* and *Allium*, therefore, that the absence of accessory nuclei is proof that irradiation took place after the last division does not apply to my material. Fragments that lie somewhat apart from the chromosomes are enclosed in small accessory nuclei (Fig. 5). In one embryo containing 52 neuroblasts in middle and late telophase the fragments are contained in 59 accessory nuclei and in 10 of the cell nuclei. Thirty-seven per cent of the former and 40% of the latter exhibit a stainability comparable to that of normal chromosomes, while the remaining 63 and 60%, respectively, are pyknotic, resembling in this respect the more deeply staining chromosomes of the ganglion cell nucleus (Fig. 6). There are also intermediate types in which one end of the fragment lies out in a small accessory nucleus, while the other end lies within the cell nucleus and appears to have become joined to the distal end of a chromosome (Fig. 4). Of the 59 accessory nuclei and 10 cell nuclei referred to above, 30% of the former and 60% of the latter show connections between fragments and chromosomes. This suggests delayed attachment.

5. *Interphase*.—Fragments persist throughout the interphase as dark staining elements contained within either accessory nuclei (Fig. 6) or cell nuclei, depending on their final location at late telophase.

Fragments and the Mechanism of Mitosis.—Any hypotheses bearing on the mechanism of mitosis must not overlook certain parallels that are manifest in the behavior of chromosomes, which possess spindle attachments, and fragments, which lack them. The facts must not be disregarded that (1) the fragments lie in the equatorial plane at metaphase, (2) their "chromatids" begin to separate at anaphase at the same time as do those of the chromosomes, (3) their "chromatids" come into intimate contact with the spindle at middle anaphase, and (4) sister "chromatid" fragments usually move toward opposite poles behind the chromosomes and so are included at telophase in different daughter cells.

(1) Many cytologists who have studied the mechanism of mitosis have been inclined to view the movement of the chromosomes into the equatorial plane at the end of the prophase as a force of some kind exerted by the spindle—in conjunction with the poles—on the chromosome through its spindle attachment. The regularity with which fragments, which lack spindle attachments and have no contact with the spindle, come to lie in the equatorial plane at metaphase, while it does not disprove the existence of an influence exerted by the spindle through the spindle attachment of the chromosome, nevertheless does demonstrate that other factors may be involved. To account for the orientation of the chromosomes in the equatorial plane at metaphase, Lillie⁴ developed the hypothesis that the poles and chromosomes are electronegative, while the mid-region of the spindle is electropositive, the metaphase orientation resulting from the equilibrium established between these repelling and attracting forces. Darlington⁵ holds that "the arrangement on the metaphase plate must be due to repulsion from the poles acting on the centromeres." Repelling forces, whatever their nature, between poles and chromosomes and between chromosomes *inter se* seem to offer the most reasonable explanation of the metaphase location of fragments. Forces must not be limited, however, to an action through the spindle and spindle attachments, since the fragments lie outside the spindle and lack spindle attachments. Their location in the peripheral part of the metaphase plate outside the other chromosomes suggests polar and interchromosomal repulsions, while their failure to lie within the spindle is doubtless due to the absence of spindle attachments. During cytokinesis vortical currents of the protoplasm are known to pass from the poles to the equatorial plane near the cell periphery, inward at the equator and poleward along the spindle. Currents at metaphase in the outer region of the cell moving toward the equator might carry fragments to the equatorial region, where they would

be held in the slower moving or stationary protoplasm among the distal ends of the chromosomes and between them and the cell membrane. It is a question, however, to what extent currents are present at metaphase. Bělař⁶ stated that in the grasshopper spermatocyte they usually begin at early anaphase, though sometimes earlier or later than this. I have no evidence regarding this in the grasshopper neuroblast.

(2) Bělař,^{6,7} Bleier,⁸ Schaede⁹ and Schrader¹⁰ have come to the conclusion, from observations of a variety of material, that at least the initial separation of chromosomes is autonomous. The separation of the fragments, which occurs simultaneously with that of the chromosomes, demonstrates conclusively that, at least in these neuroblasts, the initial separation of "chromatids" can occur even though a spindle attachment region and a connection with the spindle are absent. This indicates, then, unless one assumes that other outer forces in the cell are the effective factors, that the forces causing chromatid separation reside within the chromosome itself, and so the act is autonomous. This evidence is not in accord with the views of Mather and Stone,¹¹ Darlington,⁵ Upcott¹² and others, who hold that the anaphase separation of chromatids is invariably determined by the division and mutual repulsion of the spindle attachment bodies at the end of the metaphase.

(3) Fragments appear to be pushed against the spindle as a result of the decrease in equatorial diameter of the cell that accompanies its axial elongation at anaphase. This movement may be aided by protoplasmic currents passing inward at the equator. Just what connection is finally established between fragment and spindle is difficult to determine. While daughter chromosomes at anaphase have their distal ends rounded and their proximal ends pointed, as if they were continuous with a spindle fibre, both ends of daughter fragments frequently show an encircling fringe of dark-staining material, resembling, though perhaps only superficially, the ends of the anaphase meiotic chromosomes described by Schrader in *Protortonia* and by Hughes-Schrader in *Llaveia*.¹³ The "Stemmkoerper" hypothesis of Bělař^{6,7} seems to offer the most satisfactory explanation of such structures in fragments. When daughter fragments that have separated get among the outer fibres of a "Stemmkoerper" that is actively elongating and therefore tends to exert axial forces and lateral pressure against the sides of these elements, the edges at their ends might be pushed outward as encircling fringes. It seems improbable that any more of a connection between fragment and spindle exists than a close contact, which is effective in altering the position or shape of the fragment only in so far as there is pressure and friction between the two.

(4) Once the fragments have come in contact with the spindle, their tendency to become oriented parallel to its axis, their final separation

and their subsequent movement away from each other in the direction of the poles, may possibly be due, at least in part, to currents passing poleward. The hypothesis proposed by Schaede⁹ could account for this behavior, since, according to it, poleward moving streams of protoplasm within the spindle are assumed to carry the daughter chromosomes to the poles. Vortical currents passing from the equator along the outer sides of the spindle to the poles might have a part in the poleward movements of daughter fragments not lying entirely within the spindle. Bělař,⁶ who investigated the relation of these to chromosome movement, came to the conclusion, however, that the anaphase movements of the chromosomes are entirely independent of such currents. He attributed the middle and late anaphase movements of the daughter chromosomes to the elongation of the "Stemmkoerper." The poleward movement of fragments, if it is not the result of currents, supports Bělař's hypothesis at the same time that it is at variance with hypotheses positing only a pulling action of the spindle fibres; for it seems less likely that these elements, which apparently have no true attachment to the spindle, should be pulled toward the poles by contracting spindle components than that they should be pushed along because of their contacts with an elongating "Stemmkoerper." Bleier⁸ attributed the whole anaphase movement of the daughter chromosomes to repelling forces of some kind originating in the chromosomes. It is true that this could account for the anaphase movements of fragments, though it fails to explain why the daughter fragments become oriented parallel to the spindle axis and delay their poleward movement until they come in contact with the spindle. The same difficulty confronts hypotheses of anaphase movement based on the presence of attracting forces between the poles and the chromosomes.

The main difference in the behavior of chromosomes with attachments and fragments without them is that the former move toward the poles more rapidly, and their points of attachment to the spindle lie at all times in a plane at right angles to the spindle axis, while the daughter fragments move toward the poles more slowly and with less regularity, and never arrive at a point as near the poles as the normal chromosomes. The conclusion seems justifiable, therefore, that in these cells, at least, the functions of the kinetochores are primarily to make uniform the orientation of the chromosomes in the equatorial plane at metaphase and to synchronize the middle and late anaphase separation of daughter chromosomes in order to insure their equal apportionment to the daughter cells, and not to effect their initial anaphase separation.

Delayed Effects.—In another paper² I have demonstrated that broken ends of chromosomes possessing spindle attachments can be transmitted, through the formation and breaking of chromatin bridges, to the second

cell generation following their production. It has been shown in the present paper that daughter fragments may be included at telophase in the cell nucleus with the chromosomes. McClintock¹⁴ has demonstrated in *Zea* that broken ends of chromosomes retain their tendency to fuse and undergo unions from generation to generation. If the same is true of fragments, all the chromosomal conditions necessary for the occurrence of delayed attachment, as postulated by Stadler,¹⁵ are fulfilled. In the present paper cells have been described that exhibit what are probably delayed attachments between fragments and chromosomes (Fig. 4).

I have no direct evidence regarding the behavior or fate of fragments beyond the mitotic cycle in which they appear. If, failing to become attached to a chromosome during that interphase, they fail to survive the next division, delayed effects of this kind will be limited to the chromosomes of the second cell generation after irradiation. If they may persist, undergo division and be included in the cell nucleus at the end of the second division after their formation, there is no reason why they may not survive through further cell generations, with the possibility at any time of delayed attachment with chromosomes at their broken ends. Some cells show sister fragments passing into different daughter cells seven days after irradiation, and it seems likely that these are undergoing their second division after irradiation.

Helwig¹⁶ found a sufficient number of different chromosomal alterations represented in the secondary spermatogonia of single cysts, all the cells of which are descended from a single irradiated primary spermatogonium, to conclude that "fragmentation of the chromatin does not necessarily occur at the time of irradiation, but may be delayed." Up to the present time there has been no positive demonstration of delayed fragmentation except in some relatively rare cases of the breaking of a chromatin bridge in two places, so that a fragment lags at the cell equator. In this study and another,² however, I have found, in the persistence from one cell generation to the next of chromosomes and fragments with broken ends, a cytological basis for the occurrence of delayed attachments. The suggestion seems justifiable, therefore, that the delayed effects observed by Helwig might be interpreted in this way.

Summary.—The mitotic behavior of x-ray induced fragments lacking spindle attachments parallels that of the unaltered chromosomes of the grasshopper neuroblast in several respects. Sister "chromatids" of fragments separate at anaphase and are usually included in different daughter cells at telophase. Not infrequently they are included in the newly formed cell nucleus. This behavior has a bearing on certain hypotheses of the mechanism of mitosis and on the question of delayed reattachments following fragmentation.

¹ Throughout this paper the term *fragment* is used to designate a portion of a chromosome resulting from one or more breaks in an original chromosome and having no spindle attachment. The term *chromosome* is applied to an original chromosome or the part of an original chromosome containing a spindle attachment.

² Carlson, J. G., *Genetics* (in press).

³ Mather, K., *Proc. Roy. Soc. London*, B124, 97-106 (1937).

⁴ Lillie, R. S., *Am. Jour. Physiol.*, 15, 46-84 (1905); *Jour. Morph.*, 22, 695-730 (1910).

⁵ Darlington, C. D., *Recent Advances in Cytology*, Philadelphia (1937).

⁶ Bělař, K., *Arch. Entomch.*, 118, 359-484 (1929).

⁷ Bělař, K., *Naturwiss.*, 15, 725-734 (1927); *Zeit. Zellforsch. mikr. Anat.*, 10, 73-134 (1930), 17, 51-66 (1933).

⁸ Bleier, H., *La Cellule*, 40, 85-144 (1930); *Genetica*, 13, 27-76 (1931).

⁹ Schaeede, R., *Planta*, 8, 383-397 (1929); *Beitr. Biol. Pflanzen*, 19, 141-177 (1931).

¹⁰ Schrader, F., *Zeit. wiss. Zool.*, 142, 520-539 (1932).

¹¹ Mather, K., and Stone, L. H. A., *Jour. Genetics*, 28, 1-24 (1933).

¹² Upcott, M., *Proc. Roy. Soc. London*, B124, 336-361 (1937).

¹³ Schrader, F., *Zeit. wiss. Zool.*, 138, 386-408 (1931); Hughes-Schrader, S., *Zeit. Zellforsch. mikr. Anat.*, 13, 742-769 (1931).

¹⁴ McClintock, B., *Genetics*, 23, 315-376 (1938).

¹⁵ Stadler, L. J., *Proc. Sixth Internat. Congress Genetics*, 1, 274-294 (1932).

¹⁶ Helwig, E. R., *Jour. Morph.*, 55, 265-311 (1933).

A TITANOTHERE FROM THE TYPE SESPE OF CALIFORNIA

BY CHESTER STOCK

DEPARTMENT OF GEOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated October 7, 1938

Introduction.—In previous papers,¹ published mostly in these PROCEEDINGS, have appeared some of the paleontological results of explorations conducted in the Sespe deposits of Southern California. While the field efforts south of the Santa Clara Valley, Ventura County, were rewarded by rather startling results, no small amount of irritation was felt because of failure to find fossil vertebrate remains in the Sespe at the type locality north of the Santa Clara Valley. For, as is now known, the Sespe is not of same age throughout its stratigraphic thickness or at the several localities where fossil mammals have been found in it. It is, in fact, a series of beds that range in age from at least the upper Eocene to apparently the lower Miocene. Thus it seems especially important to determine by means of vertebrate evidence the age relationships of the type Sespe on Sespe Creek to that portion of the Sespe whose age is already established south of the Santa Clara Valley.

With this problem in mind the rugged terrain north of the Ojai Valley,

Ventura County, and in the immediate vicinity of Sespe Gorge was critically examined during the summer of 1937 by a field party of the California Institute of Technology. The outcome of this work was the discovery of titanotheres remains in the lower portion of the Sespe, including a palate with teeth permitting a comparison with the titanotheres known from lower Tertiary horizons of southern California.

Geologic Occurrence.—The titanotheres remains were encountered in massive gray-brown to pink sandstones of the Sespe on Sespe Creek (Plate 1). These heavily bedded and well indurated strata are conformable to the sandstones and shales of the marine upper Eocene. The uppermost marine formation in contact with the Sespe has been recognized as the Coldwater on the basis of invertebrate fossils and stratigraphic position by F. E. Dreyer,² who, prior to the discovery of the vertebrate remains, had studied the geology of the region. Deformation and structural complications in the immediate vicinity of the fossil locality make it difficult to determine the exact position of the titanotheres remains in the Sespe above the contact with the Coldwater. However, it can be stated with some assurance that the location is not less than 400 feet nor more than 700 feet above the base of the Sespe.

DESCRIPTION OF MATERIAL

Teleodus cf. californicus Stock

Specimen.—A portion of a skull representing principally the palate with upper dentition, No. 2143 C. I. T. Vert. Pale. Coll., Plate 2, figs. A and B.

Locality.—C. I. T. Vert. Pale. Loc. 292, near north boundary of NE¹/₄, Sec. 2, R. 23 W., T. 5 N., San Bernardino B. & M., Mt. Pinos Quadrangle, Calif.

Comparisons.—The specimen, No. 2143, represents a mature individual in which the first molar and anterior premolars show considerable wear. The incisors and canines are likewise worn.

Two incisors are present on either side of the median line and these teeth possess the rounded, non-cingulate crowns seen in *Teleodus californicus*.³ The lateral incisor is larger than the medial one. The canines possess relatively small crowns. This tooth resembles the canine in *T. californicus*, but the crown is actually of smaller size. A slightly longer diastema is present in No. 2143 than in No. 1834 C. I. T. Vert. Pale. Coll., from the Sespe uppermost Eocene beds (C. I. T. Vert. Pale. Loc. 150) north of the Simi Valley. Unfortunately the premolars are considerably worn. *P*₁ is not present on the right side and but poorly preserved on the left. What remains of the tooth suggests a triangular shaped crown and a size like that in *T. californicus*. It is possible that *P*₁ was slightly larger in No. 2143 than in individuals from locality 150. Resemblance between the

latter species and the present specimen is seen likewise in the size and shape of the posterior premolars.

Comparison of the molars in No. 2143 with those of *T. californicus* from the Sespe north of the Simi Valley reveals again a close similarity between these two forms in size, shape and in details of structure of the tooth crowns. As in the latter species the hypocone is distinct in *M*₃.

Comparing No. 2143 with a skull of *Teleodus uintensis*, No. 8634 Car-

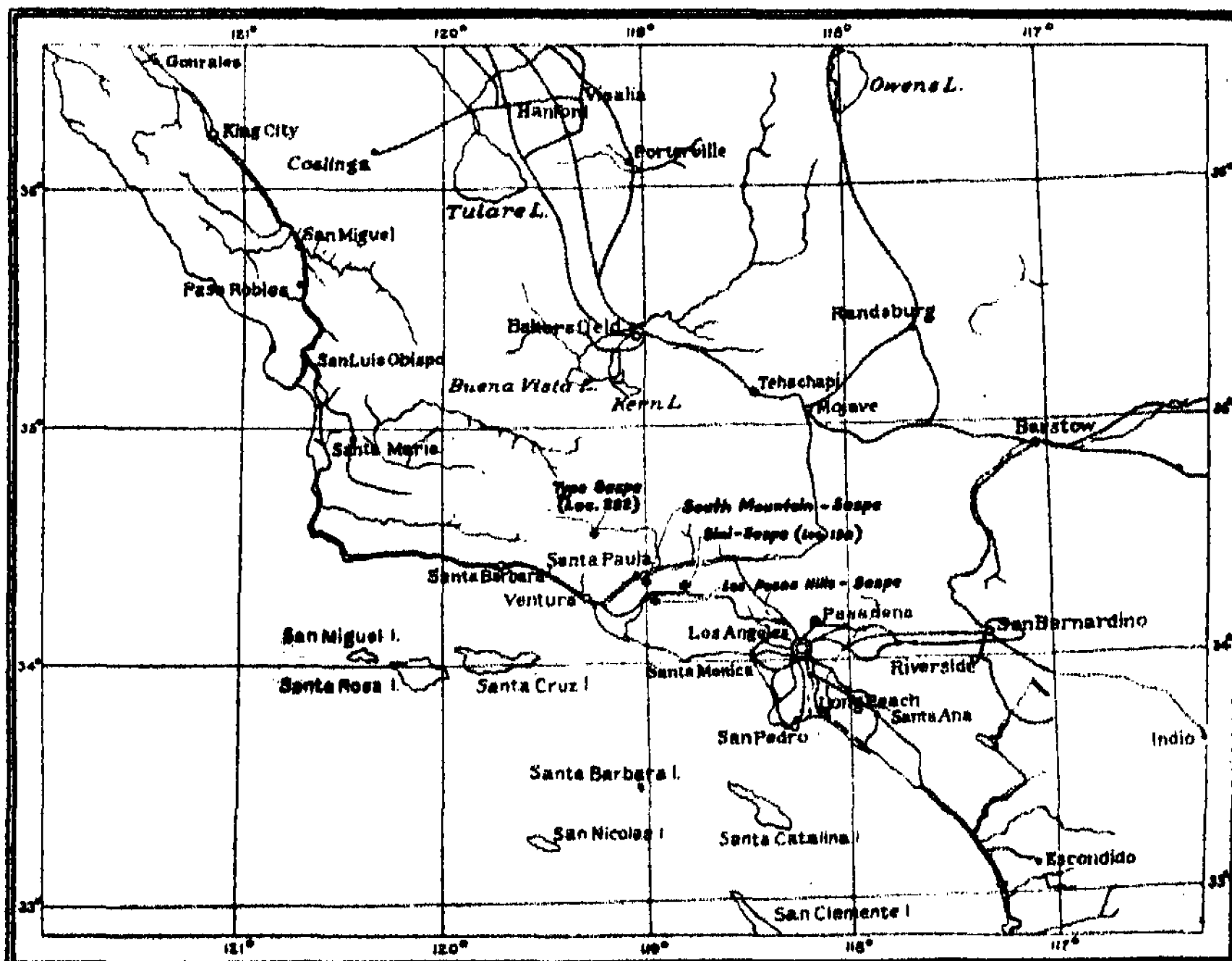


FIGURE 1

Index map showing location of principal vertebrate fossil occurrences in Sespe deposits of California. Note particularly position of localities 292 and 150.

negie Museum, from the Duchesne River formation of Utah, it is seen that certain differences in proportion exist between the two. Thus, while there is resemblance in width of snout across the canines and to some extent also in the length of the tooth-row, *C-M*₃ inclusive, the distance between the border of the maxillo-nasal notch and the anterior border of the orbit is shorter and the orbit is smaller in the California specimen.

No. 2143 is distinctly smaller than *Protitanops curryi* from the Titus Canyon formation, California. In the presence of larger incisors and in the shape of the anterior premolars the Sespe form is less advanced than the latter.

Conclusions.—A comparison of the titanotheres remains from locality 292 with those previously described from the Californian area suggests a close similarity in stage of evolution to *Teleodus californicus* from the Sespe north of the Simi Valley. The differences between these two forms may be established ultimately as of specific value but their importance on the basis of available material does not appear to be greater than that resulting from variation within a single specific group.

In the light of this similarity one may conclude that that portion of the Sespe in which locality 292 occurs north of the Santa Clara Valley is



PLATE 1

View looking northeast across Sespe Creek showing typical exposure of heavily bedded sandstones of the Sespe continental beds resting on the Coldwater marine Eocene, and locality where titanotheres was found.

closely related in time to the stage of the Sespe containing the fauna found at locality 150 south of the Santa Clara Valley. The assemblage of fossil mammals found at the latter locality has been regarded as of uppermost Eocene age. Its relationships are with the Duchesne River fauna of Utah and its position in the faunal sequence of western North America is between the Uinta (Uinta C) upper Eocene and the White River lower Oligocene.

Underlying the Sespe in the region of locality 292 is the marine Coldwater and the latter has come to be regarded as upper Eocene in age. In fact, W. P. Woodring⁴ considers the Coldwater of the western Santa Ynez

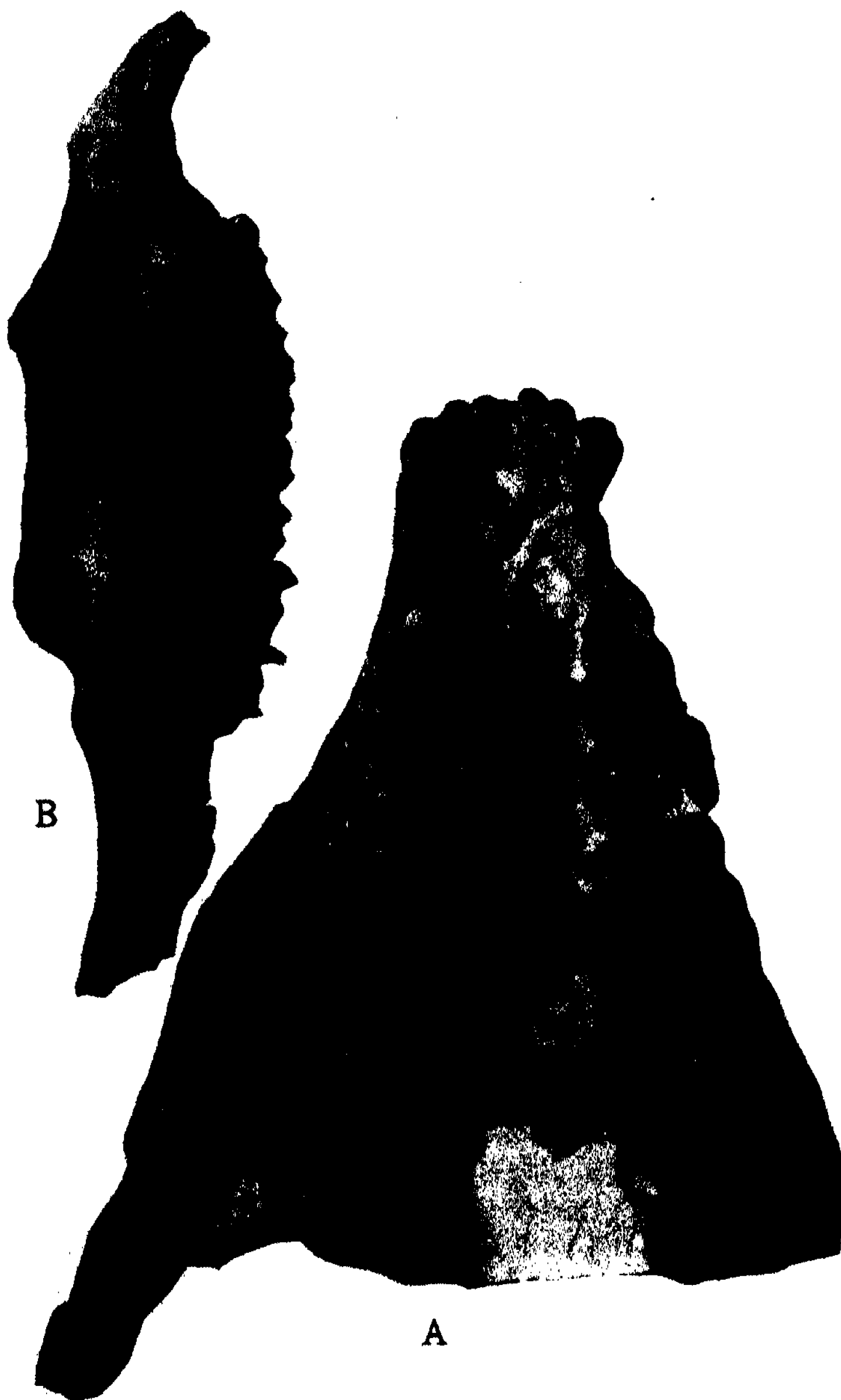


PLATE 2

Teleodus cf. *californicus* Stock

Figures A and B, skull fragment with upper dentition, No. 2143 C. I. T. Vert. Pale. Coll. figure A, $\times 1/3$; figure B, slightly smaller than $1/3$ natural size. Sespe Uppermost Eocene, California.

Range as representing the youngest Eocene faunal zone in California. Thus, the age determination of the Sespe at locality 292 as uppermost Eocene does not conflict with the evidence furnished by the marine invertebrates in the Coldwater and by the conformable relationship of the latter deposits to the Sespe. Since the Sespe is considerably thicker not far to the east of the section in which the titanotherium was found, it is reasonable to regard the age of these continental beds as extending from the uppermost Eocene into at least the Oligocene.

MEASUREMENTS (IN MILLIMETERS) OF No. 2143

Width measured across outer sides of canines		68.8
Length of diastema between <i>C</i> and <i>P</i> ₁		19
Length from anterior end of <i>C</i> to posterior end of <i>M</i> ₃		283
Medial incisor, width		9.2
Lateral incisor, width		10.4
Canine, width	15.8;	length 19
<i>P</i> ₂ , transverse diameter	<i>a</i> 25.5;	length through middle <i>a</i> 17.6
<i>P</i> ₃ , transverse diameter	31.7;	length through middle 23.6
<i>P</i> ₄ , transverse diameter	41.9;	length through middle 29.7
<i>M</i> ₁ , transverse diameter	45 ;	length through middle 39
<i>M</i> ₂ , transverse diameter ⁴	55 ;	length through middle 52
<i>M</i> ₃ , transverse diameter	61.7;	length through middle 61.4
Distance from anterior end of snout at point between medial incisors to anterior border of orbit		164
Depth of zygomatic arch immediately in back of postorbital process		51.6
<i>a</i> , approximate.		

¹ Stock, C., see these PROCEEDINGS, 1932-1935.

² Dreyer, F. E., Thesis entitled: "The Geology of a Portion of Mt. Pinos Quadrangle, Ventura County, California," on file at University of California at Los Angeles.

³ Stock, C., *Proc. Nat. Acad. Sci.*, 21, 456-462 (1935).

⁴ Woodring, W. P., *Trans. San Diego Soc. Nat. Hist.*, 6, 386 (1931).

AUTOSOMAL LINKAGE IN MAN—THE RECOMBINATION RATIO BETWEEN CONGENITAL TOOTH DEFICIENCY AND HAIR COLOR¹

BY BARBARA S. BURKS

CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, LONG ISLAND

Communicated October 14, 1938

In the course of a search for linked traits, data have been obtained which establish for the first time a clear instance of autosomal linkage² in man, provide an estimate of the recombination ratio, and at the same time

elucidate the modes of inheritance of the two traits involved—tooth deficiency and hair color. A brief note on this study was published in 1937 (3). The present paper is based upon further data, and furnishes a fuller statement of material obtained and analysis used.

The method employed for analyzing the data is an adaptation of a technique used earlier by the author. Although the statistical devices proposed by Bernstein in 1931, by Wiener in 1932 and by the author in 1932, have been advanced and refined through the theoretical work of the University of London group (Fisher, Haldane, Hogben, *et al.*), who have provided precise and efficient techniques for detecting linkage and estimating the amount of crossover, the newer methods have required in general:

(1) that the genetic formulae of the parents were either known or could be inferred,

(2) that the modes of transmission of the traits to be studied were well understood,

(3) that the method of ascertainment of cases was known. There are many instances in which none of these conditions hold; when, in fact, the detection of linkage relationships might serve to *clarify* the transmission mechanisms. As this was true of the data to be reported herewith, we reluctantly turned back from the London formulae, and dusted off our earlier study (Burks and Tolman, 1932). Although the results of this investigation had been negative, the method had the advantage of being applicable to complex traits, and of requiring for experimental subjects the members of only a single generation. Briefly, the method involved the comparison of pairs of siblings upon one trait when (*a*) they resembled each other, and (*b*) did not resemble one another upon a second trait or group of traits. If two traits showed no correlation in the general population, but did tend to vary concomitantly within a sibship, evidence for linkage could be adduced.³

Eugenics Record Office Study.—Following a clue from a three-generation pedigree published by Beadle in 1926, the indexed repository of family schedules at the Eugenics Record Office was searched for cases showing congenital tooth deficiency.⁴ Fifteen usable families were found (with at least one affected sibling, and with two or more siblings past the age range in which rapid changes in hair pigmentation are taking place—i.e., past fifteen). Six categories ranging from black to flaxen were employed in these schedules for recording hair color; also three categories of red, which occurred only twice in this material, and was disregarded in the analysis because of its genetic independence of brown or melanic pigment (5).

Members of each sibship were paired in all possible combinations, and the pairs so obtained were arranged fourfoldwise according as the paired offspring were similar or dissimilar in each of the two traits under considera-

tion. Following Mather (6), these separate familial tabulations were combined without weighting. The chi-square technique was applied for testing the significance of deviations from proportionality of the combined fourfold tables. The Boas-Yulean ϕ was also computed as a rough indicator of *closeness* of association.

The basic results for tooth deficiency and hair color are shown in table 1. Subdivision was made of (1) ten families in which a parent and at least one offspring were affected, and (2) the remaining five families. It is reasonable to suppose either that families with and without an affected parent are genetically non-homogeneous with respect to each other, or that the reports

TABLE 1

TOOTH DEFICIENCY TESTED FOR LINKAGE WITH HAIR COLOR: EUGENICS RECORD OFFICE CASES

GROUP	NO. OFFSPRING		NO. PAIRS	ϕ	CHI-SQUARE	P	HAIR	TOOTH DEFICIENCY	
	AF- FECTED	NORMAL						SIMILAR	DIS- SIMILAR
15 families, 1 or more af- fected off- spring	28	26	88	0.17	1.75	<0.20	Sim. Dissim.	14 34	6 34
10 families, parent and 1 or more affected off- spring	22	13	56	0.21	1.47	<0.24	Sim. Dissim.	8 21	3 24
5 families, nei- ther parent affected	6	13	32	0.09	0.02	0.90	Sim. Dissim.	6 13	3 10

* Yates's correction for continuity has been applied.

are more accurate in the group designating affected parents as well as offspring. For whichever reason, the tests for linkage in the two subgroups give quite different results.

The evidence as a whole appears positive for linkage, i.e., the fourfold tables deviate from proportionality in the expected direction in the case of the total group, and in the subgroup of ten families having an affected parent. In evaluating the probabilities that in fourfold distributions of independent variables, chance deviations would occur as large as those actually found, it must be borne in mind that fluctuations in a direction *favorable* to linkage would occur decidedly less often than the *P*'s indicate—by the order of approximately one-half. The results, while not conclusive, seemed to justify collection of further data.

Field Study.—To enlarge the preliminary study based upon available records, additional families with affected offspring were located through

clinics and interested persons. Care was taken in the selection of proband cases to secure a group whose missing teeth (incisors or premolars) were almost surely congenitally missing, and not merely extracted, impacted or retarded.

Out of forty-three referred families that could be located and visited, there were fourteen containing two or more siblings over fifteen years of age. When individuals in these families, by inspection, lacked one or more teeth (not accounted for by extraction), confirmation was sought by x-ray. Third molars were at first disregarded entirely, but when chance observations suggested that missing third molars might be genetically related to other missing teeth, and possibly furnish an explanation of apparently "isolated" or non-familial cases of tooth deficiency, they were included thereafter in the diagnostic schedule. In the fourfold tabulations of table 2,

TABLE 2

TOOTH DEFICIENCY TESTED FOR LINKAGE WITH HAIR COLOR: FIELD CASES

GROUP	NO. OFFSPRING		NO. PAIRS	ϕ	CHI- [*] SQUARE	P	HAIR	TOOTH DEFICIENCY	
	AFFECTED	NOR- MAL						SIMI- LAR	DISSIMI- LAR
14 families, 1 or more affected offspring	36	16	106	0.22	4.05	<0.05	Sim.	37	8
							Dissim.	38	23
10 families, normals surely differentiated from cases with missing third molars (1 family overlaps with following subgroup)	29** (+2)	5	63	0.25	2.77	<0.10	Sim.	29	3
							Dissim.	22	9
5 families, third molars not diagnosed in otherwise normals (1 overlapping family)	7 (-2)	11	43	0.08	0.026	0.90	Sim.	8	5
							Dissim.	16	14

* Yates's correction for continuity has been applied.

** Of these, 13 cases were affected only in third molars.

affected sibling pairs were counted "similar" regardless of which or how many teeth were congenitally missing.

Hair color was appraised by matching the hair of the back of the head, close to the scalp, against the standard "Shur-on" chart of human hair. We followed the principle of classifying sibling pairs as "similar" in hair color if their shades were so close together on the chart that discrimination between the two shades of hair was doubtful or even liable to a reversal of direction if repeated on another day. All other sibling pairs were counted "dissimilar."

The relation between tooth deficiency and hair color in these families is shown in table 2. The evidence for linkage is somewhat stronger in this array of data than in that based upon the records—in part because a larger number of sibling pairs was available. Again we recall that the prob-

ability that chance deviations from proportionality should be in a direction *favorable* to linkage is of the order of one-half the P value corresponding to chi-square. The two independent sets of data can leave little doubt as to the actual presence of linkage.

Estimate of Recombination Ratio.—In reaching an estimate of the recombination ratio, it is necessary to adopt some reasonable hypothesis as to hereditary mechanisms that will not only account for the sibling distributions of the two traits, but for the *linkage relation* between them. With the two traits under discussion, there is no common agreement as to their modes of transmission. Bearing in mind the different methods by which

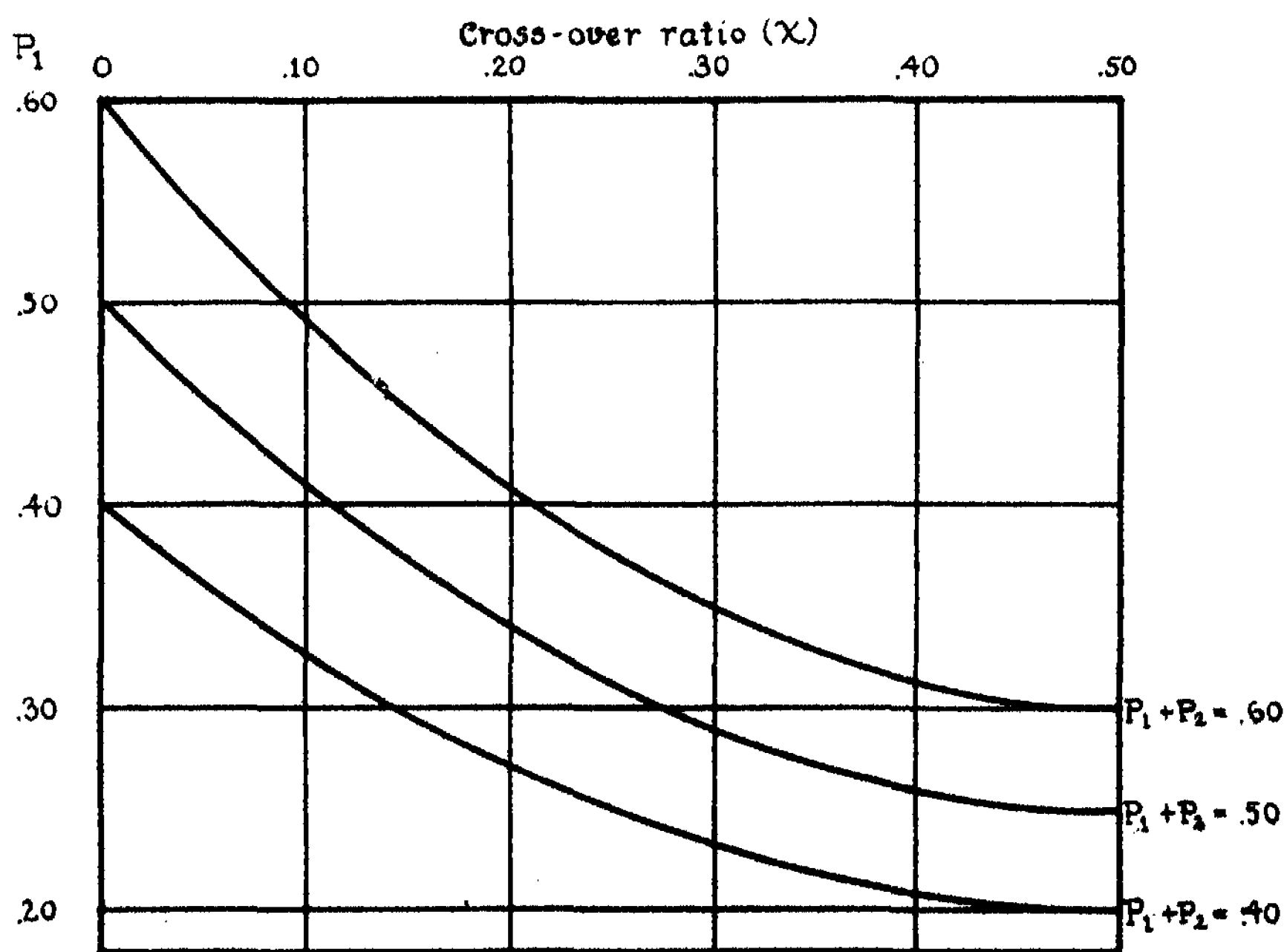


FIGURE 1

Calculated cross-over ratio for given combinations of P_1 and P_2

the E. R. O. cases and the field cases were ascertained and the data collected, the numbers of affected⁶ and normal offspring, the proportions of similar and dissimilar pairs on each trait, and the *differences* in similar pairs on trait 1 in comparisons of similar and dissimilar pairs on trait 2 (and vice versa), we may investigate the consequences of the following genetic schema, which fits the data better than any other we were able to devise:

Tooth deficiency: missing third molars due to simplex condition Aa ; more extensive deficiencies due to duplex condition AA ; occasional overlap in phenotypes; selective but

irregular suppression of the different teeth comparable to the influence of *Dichaete*, *Hairless*, *Scute* or *Echinus* in suppressing particular bristles in *Drosophila* (8).

Hair color: varied and widely distributed series of alleles incompletely dominant with respect to one another, giving (1) in families with two heterozygous parents, four potential phenotypes corresponding to four genotypes [H_1H_3 , H_1H_4 , H_2H_3 , H_2H_4] and (2) in families with one homozygous parent (or families in which certain phenotypes are not readily distinguished), two potential phenotypes corresponding to two genotypes [H_1H_2 , H_1H_3].

In table 1, the subgroup for which an affected parent is reported presumably arises from matings $AA \times Aa$. Removing the record of one atypical family sent in by an interested correspondent *because* of the large number of affected members (mother and six of seven offspring), we have

the fourfold $\begin{matrix} + & T & - \\ H & +7 & 2 \\ & -7 & 19 \end{matrix}$ which would seem to conform to hair color plan 1.

According to these assumptions, the following equations furnish an estimate of the recombination ratio, χ . For pairs similar in tooth deficiency, proportion similar in hair color,

$$P_1 = \frac{1}{2}[\chi^2 + (1 - \chi)^2] \quad \text{whence } \chi = 0.$$

For pairs dissimilar in tooth deficiency, proportion similar in hair color,

$$P_2 = \frac{1}{2}[2\chi(1 - \chi)] \quad \text{whence } \chi = 0.120.$$

Combining above equations,

$$P_1(2\chi - 2\chi^2) - P_2(2\chi^2 - 2\chi + 1) = 0 \quad \text{whence } \chi = 0.096.$$

Values of χ corresponding to different combinations of P_1 and P_2 have been calculated from the third equation, and are shown in graphical form in figure 1. It should be noted that diagnosis of third molar cases (i.e., heterozygous cases) is not necessary for estimation of χ in this group if our hypothesis is tenable, since all offspring not AA would then be Aa . Even if our hypotheses of simplex and duplex phenotypes should have to be enlarged to include *some* pedigrees in which the simplex condition is sufficient to produce missing anterior teeth, these pedigrees would contain only two types of offspring (Aa and aa), and the formulae for estimation would remain the same.

The field cases offer additional complications. There is a paucity of information regarding the parent generation, for most of the parents (in the underprivileged groups located through clinics) had lost so many teeth by decay that we could seldom determine whether any were congenitally missing or not. A rough estimate of χ is possible, however. The distributions suggest that the majority of families may be of an $Aa \times Aa$ type of mating, and that the diagnosis of missing third molars in the offspring is

therefore of particular importance. Plan 2 appears to fit the data on hair color better than plan 1, possibly in part because of a different racial composition of families, and in part because our method of classification was likely to merge fine differences.

Using the subgroup in which third molars were diagnosed, we may calculate the numbers of sibling pairs who are presumably $AA-AA$, $AA-Aa$, $Aa-Aa$, according to our hypothesis, and from these, the proportion similar in hair color to be expected among all affected pairs for different values of χ . The proportion, 0.588, in the present material would correspond to χ between 0.10 and 0.15.

The same procedure might equally well be followed with the sibling pairs having one affected and one normal member. The present data are hardly numerous enough to justify this calculation, though it is interesting to note that the data as they stand correspond to an estimated recombination ratio between zero and 0.05. The fluctuations of these various estimates are no greater than sampling errors could account for, and indeed the general *convergence* of estimated values of χ from the different data of differing genetic composition lends strong support to the genetic formulation advanced.

Further investigation may possibly require modification of our genetic interpretation, with a shift in estimated χ . The present data considered, it seems probable that the actual recombination ratio lies not far from 0.10.

REFERENCES

1. Beadle, O. J., *Genetics*, 17, 199-206 (1926).
2. Bernstein, F., *Zeit. induct. Abstamm. Vererb. Lehre*, 57, 113-138 (1931).
3. Burks, B. S., *Yearbook Carnegie Inst. Wash.*, 36, 312-319 (1937).
4. Burks, B. S., and Tolman, R. S., *Jour. Genet. Psychol.*, 40, 3-15 (1932).
5. Davenport, G. C., and Davenport, C. B., *Amer. Nat.*, 43, 193-211 (1909).
6. Mather, K., *The Measurement of Linkage in Heredity*, New York, Chem. Publ. Co. (1938).
7. Penrose, L. S., *Ann. Eugenics*, 6, 133-138 (1935).
8. Plunkett, C. R., *Jour. Exptl. Zool.*, 46, 181-244 (1926).
9. Wiener, A. S., *Genetics*, 17, 335-350 (1932).

¹ Grateful acknowledgment is made for counsel and facilities provided by Drs. C. B. Davenport and H. H. Laughlin of the Eugenics Record Office. Dr. John O. McCall, director of the Murry and Leonie Guggenheim Dental Clinic opened the files of the clinic to our field workers, and generously provided dental x-rays of patients and members of their families whom we referred. Through Dr. William Hemley, director of the Orthodontia Clinic of New York University Hospital, several additional families were secured for investigation. Members of the staffs of both clinics showed us every courtesy and consideration, as did the dentists in private practice to whom we referred cases for x-ray. The writer also expresses appreciation for the contributions of Mrs. Jean C. Challman and Miss Natalie M. Raymond who assisted in gathering the field data.

² The possibilities of sex linkage or incomplete sex linkage, or of correlation between tooth deficiency and hair color were excluded at the outset by appropriate tests.

³ Penrose (7) has independently proposed a similar scheme. The present data have been tabulated in the "fourfold table" form employed by Penrose, but the treatment of data has varied from his, and from our earlier treatment, in a number of respects.

⁴ The teeth most frequently reported as missing were the permanent upper lateral incisors, though lower incisors and upper or lower pre-molars were often missing either singly or in various combinations. Affected offspring in individual families often varied among themselves in the location and degree of involvement. Only one individual was reported as having missing third molars (this being in conjunction with other tooth deficiencies). We have reason to believe that missing third molars often pass unnoticed.

⁵ To guard against any possible bias, family records of hair color, with names and notations on teeth detached, were submitted to Jean C. Challman, whose classification, corroborated by the writer, was used in the final tabulation.

⁶ The data of the first subgroup in table 2 were further subdivided to demonstrate the common genetic derivation of missing third molars and other missing teeth. The number of cases is not large enough to establish the proportions with great accuracy, but pairs in which both members are affected, regardless of which teeth, appear to be definitely more similar in hair color than pairs having one affected and one normal member. For this table chi-square = 8.8014, $N = 4$, P is between 0.10 and 0.05.

	BOTH LACKING TEETH OTHER THAN THIRD MOLARS	BOTH LACKING THIRD MOLARS	1 LACKING "OTHER" 1 LACKING MOLARS	1 LACKING "OTHER" 1 NORMAL	1 LACKING MOLARS 1 NORMAL
Number of pairs	15	17	19	7	5
Proportion similar in hair color	0.40	0.76	0.58	0.29	0.20

REMARKS ON PLATEAU'S AND DOUGLAS' PROBLEM

BY R. COURANT

DEPARTMENT OF MATHEMATICS, NEW YORK UNIVERSITY

Communicated October 7, 1938

The objective of Plateau's Problem in the generalized form by J. Douglas¹ is the proof of the existence of a minimal surface of given topological structure and with k prescribed boundary curves. Its solution has been the subject of several recent publications.² Therefore it may be justified to give the following account of a different and simplified version of the existence proof contained in a forthcoming paper on this and related subjects.

Minimal surfaces in the n -dimensional space with the coördinates x_1, \dots, x_n or the position vector \mathbf{r} are represented by functions x_i of two parameters u, v in a domain B over the plane of the complex variable $w = u + iv$, in such a way that the x_i are harmonic in B :

$$\Delta \mathbf{r} = \mathbf{r}_{uu} + \mathbf{r}_{vv} = 0$$

and that $\xi_u^2 - \xi_v^2 = \xi_u \xi_v = 0$. Since for harmonic vectors ξ the expression $\varphi(w) = \xi_u^2 - \xi_v^2 - 2i\xi_u \xi_v$ is analytic in w this second condition is simply represented by

$$\varphi(w) = \Sigma f'_\nu(w)^2 = 0.$$

Here $f_\nu(w)$ denotes the analytic function whose real part is x_ν . We now consider domains B of the prescribed topological structure. The boundary C of B consists of k curves C_1, \dots, C_k which will be made to correspond monotonically to the k prescribed curves $\Gamma_1, \dots, \Gamma_k$ in the x -space.

The solution of the Plateau Problem is obtained by solving the following variational problem: Let $\xi(u, v)$ be a vector continuous in $B + C$ which maps C , in monotonically on Γ , has piecewise continuous first derivatives in B and for which the Dirichlet Integral

$$D[\xi] = \int \int (\xi_u^2 + \xi_v^2) du dv$$

exists.

It is required to find B and ξ so that $D[\xi]$ attains its lower limit d . In principle B could range over the class of all Riemann domains of the prescribed topological structure. But to avoid use of theorems on conformal mapping one restricts B to a class with smooth boundary curves, but still suitably general. In previous publications I have chosen plane domains B , e.g., for genus zero, domains bounded by k circles, or planes with k finite parallel slits; for higher topological structure slit domains were used, or fundamental domains of a Schottky group from which k circular discs are removed.³

In this paper, instead, we consider Riemann domains B with more sheets over the w -plane so that each boundary curve C_i is the unit circle. In particular, for the genus zero B may consist of k unit circles connected by means of branch points of total multiplicity $2k - 2$, e.g., $2k - 2$ simple branch points, so that each circular disc is connected with its "next" by a "branch line."⁴ If the genus is p we affix to such a domain p different full planes, each by means of 4 simple branch points. But, of course, all limiting cases, hence branch points of higher order, must be admitted. For the case of non-orientability one can easily construct similar normal domains by considering the corresponding orientable double surface.

The solution of the Plateau Problem consists of two steps. First we have to show that the variational problem has a solution—which because of the Dirichlet Principle must necessarily be a harmonic vector ξ . Secondly the relation $\varphi(w) = 0$, i.e., the character of the solution as a minimal surface must be proved.

The first step can be achieved and at the same time the lower semi-continuity of the lower bound d in its dependence on Γ , be proved under the condition: no minimizing sequence of vectors \mathfrak{x}_m for which $D[\mathfrak{x}_m]$ tends to the lower limit d can tend to degeneration. Tendency to degeneration means that there exists on the surface \mathfrak{x}_m a closed curve L_m which, on \mathfrak{x}_m , cannot be continuously deformed into a point and whose diameter tends to zero with m tending to infinity. This condition insures for a minimizing sequence of domains B_m and vectors \mathfrak{x}_m the existence of a limiting domain B of the same type and at the same time the equi-continuity of the boundary values of \mathfrak{x}_m ; the proof then proceeds without difficulty. The above condition is shown to be equivalent to sufficient conditions in form of inequalities used by Douglas and the author. This is done by means of the following lemma: If there exists a minimizing sequence tending to degeneration there also exists such a sequence all of whose members are actually degenerated; the curves L_m then are replaced by single points.

The advantage of our choice for B appears in the second step, the proof of $\varphi(w) = 0$ for the solution \mathfrak{x} of the variational problem. First, by variation of the boundary values we obtain as in the previous paper the result that $w^2\varphi(w)$ is regular and real along all the boundary circles C_r of B . Furthermore we observe that $\varphi(w) = \Sigma f'_r(w)^2$ has a pole of order not higher than $2r$ in a branch point of order r . For, if the neighborhood of such a branch point, e.g., $w = 0$ is mapped by $w = \rho^{r+1}$ to the simple neighborhood of $\rho = 0$ in the ρ -plane we have

$$\varphi(w) = \left(\frac{1}{r+1}\right)^2 \frac{1}{\rho^{2r}} \sum \left(\frac{df_r}{d\rho}\right)^2$$

and $\sum \left(\frac{df_r}{d\rho}\right)^2$ is regular around the point $\rho = 0$.

The variability of B consists in the variability of the branch points, and the corresponding variational condition for the solution \mathfrak{x} , found by a simple variation, is: $\varphi(w)$ has in a branch point of order r a pole of order not higher than r instead of the *a priori* possible order $2r$.

Now $\varphi(w) = 0$ is proved as follows: Suppose first B to be of genus zero and assume that $\varphi(w)$ does not vanish identically; then $\varphi(w)$ has in $B + C$ a finite number of zeros. N may be their—non-negative—number in B and L their number on C . The total number P of poles in B is, because of our variational condition, not greater than $2k - 2$, the total multiplicity of all branch points. The difference $N - P$ is $1/2\pi$ times the total variation of the arcus of $\varphi(w)$ if w describes the k boundary unit circles in the positive sense circumventing the L zeros of $\varphi(w)$ on C by small semicircles in B . Each of those semicircles contributes the negative amount

$-1/2$. The variation of the arcus of $w^2\varphi(w)$ along the other parts of the circles is zero because this expression is real. Subtracting the variation for w^2 , that is, $2k$ times 2π , we obtain

$$N - P = -2k - 1/2L$$

and since $P \leq 2k - 2$ we have $N \leq -2$, which is a contradiction to $N \geq 0$. Hence $\varphi(w) = 0$ is proved.

In the case of genus p the same reasoning holds. We have here $P \leq 2k - 2 + 4p$ because the right-hand side is the total multiplicity of branch points in B . Now $\varphi(w)$ is regular at the point at infinity in each of the p attached planes and has there a zero of at least 4th order, as easily seen from the definition of $\varphi(w)$. Hence *a priori* we have $N \geq 4p$, while as above $N - P \leq -2k$. This leads to the contradiction $N \leq 4p - 2$.

A similar reasoning holds for non-orientable surfaces.

In the case of genus zero the following mapping theorem results as a byproduct for $n = 2$: Every plane k -fold connected domain can be mapped conformally to a k -fold unit circle B . To establish this theorem our general theory has to be supplemented by a verification of the sufficient condition, which can be done by a reasoning similar to that on page 707 f. in the paper quoted in footnote 1.

While also for higher topological structure—not only for genus zero—our present proof proceeds without use of conformal mapping it should be pointed out that by means of mapping theorems in these higher cases a more satisfactory result is obtained—and this, as is shown in the paper quoted above, even in a simpler way. It seems as if the difficulty arising from the artificial reference to particular classes of domains of representation B cannot be eliminated otherwise. However, this difficulty does not occur if degeneration is *a priori* excluded by stronger inequality conditions, as M. Shiffman has done in his analysis of the possibility of relative minima.⁵

¹ *Bull. Am. Math. Soc.*, **36**, 50 (1930).

² 1. Douglas, *Jour. Math. and Phys.*, **15**, p. 55 ff., Feb. (1936). 2. Douglas, *Ibid.*, 105 ff., (June, 1936). 3. R. Courant, *Proc. Nat. Acad. Sci.*, **22**, 367 (June, 1936). 4. R. Courant, *Ann. Math.*, **38**, 679 (1937). 5. Douglas, *Proc. Nat. Acad. Sci.*, **24**, 343 (1938).

No. 1 gives results. No. 2 goes on to give details and proofs. No. 5 gives a summary of a detailed exposition in a forthcoming comprehensive paper. No. 3 gives two different general methods, and details for the simplest case. No. 4 contains the complete discussion of the problem for genus zero and sufficient detail for higher genus.

Douglas' method—developed since 1929—starts with a variational problem of the Dirichlet type but for harmonic functions only; this restriction permits consideration of the functional in its dependence on boundary values and thus avoids certain difficulties of the calculus of variations in two dimensions. The methods in Nos. 3, 4 and the present note are essentially based on a two-dimensional variational problem. As

shown in *Proc. Nat. Acad. Sci.*, 24, 97 (1938), they also serve to solve the problem with free boundaries. Also a forthcoming paper of M. Shiffman should be mentioned. (See footnote 5.)

³ It is preferable not to assume—as in the paper No. 4 quoted above—that these fundamental domains be bounded by circles, but rather to leave these boundaries free.

⁴ It is even permitted to assume all these branch lines parallel.

⁵ *Ann. Math.* (1938) and the forthcoming paper quoted in footnote 2. In this paper (doctoral thesis) the domain B is chosen as a slit domain as in the paper No. 4, but with the essential difference that relative minima are treated, and that the variation for the slit domains is carried out without use of conformal mapping.

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES

Volume 24

December 15, 1938

Number 12

Copyright 1938 by The National Academy of Sciences

SOME RECENT OBSERVATIONS OF SUNSPOT SPECTRA

BY HAROLD D. BABCOCK

MOUNT WILSON OBSERVATORY, CARNEGIE INSTITUTION OF WASHINGTON

Read before the Academy October 25, 1938

Powerful instruments have brought within reach of observation in the visible region a wealth of detail in the sunspot spectrum for comparison with the normal solar spectrum and with laboratory results. The most extensive discussion of this subject is found in the papers of Miss Moore,¹ whose treatment, in the light of the theory of ionization, develops a comprehensive explanation for most of the observed effects.

Corresponding observational data in the infra-red and the ultra-violet are much needed, however, since critical tests for the behavior of certain elements are found only in these regions. Indeed, the only direct evidence for the occurrence of some substances in the sun must be sought in the infra-red spot spectrum, while ultimate lines of the heavy metals lie in the ultra-violet.

The main program of sunspot spectroscopy at this Observatory has been carried out with the tower telescopes and plane-grating spectrographs on Mount Wilson. This equipment is admirably adapted for the visible region of the spectrum and can be used, though with increasing difficulty, in the more accessible part of the infra-red. But its optical limitations become practically prohibitive for the study of spots in the ultra-violet and the more difficult part of the photographic infra-red.

To supplement this combination of instruments a 21-foot concave grating spectrograph has recently been constructed at the Hale Solar Laboratory in Pasadena. The mounting is a slight modification of the Eagle type, with no optical parts except slit and grating. An achromatic solar image, either 40 cm. or 16 cm. in diameter, is provided by a reflecting telescope of 46 cm. aperture, an arrangement well adapted to the study of various solar problems. The spectrograms that have been made to test the capabilities of the equipment include a few plates showing the spectra of the prominent sunspots available in recent weeks.

The large solar image was used, with no nicol prism or other analyzing apparatus at the slit. Under these conditions an Eastman IZ plate hyper-

sensitized with ammonia requires a two-hour exposure for the spot spectrum in the region $\lambda\lambda$ 10,000–11,200, where the dispersion is about 2.4 Å/mm., while for shorter wave-lengths the time of exposure is greatly reduced. Although this dispersion is only about one-third of that given by the 75-foot spectrograph in the first order, it is sufficient for answering many questions. The omission of an analyzer is dictated at present by practical considerations, but is by no means a serious obstacle to progress even in the infra-red, partly because the dispersion is insufficient to show the resolution of the Zeeman effect in any of the spots thus far examined.

In this interesting part of the spot spectrum that is now observed for the first time there is no serious atmospheric absorption over a range of about 1000 Å to impair our scrutiny of celestial objects. It is here too that Z plates have a fairly uniform sensitivity and thus facilitate the reconnaissance. The results thus far derived from a preliminary survey of the plates are in excellent accordance with the conclusions of Miss Moore. They may be summarized in a few general descriptive statements.

Lines of the Paschen series of hydrogen are greatly weakened in the spot, as are the principal multiplets of C, O, S and P. Not much change is found for Mg. The strong Si lines are widened and moderately weakened. Definite strengthening is shown by Sr^+ , an analogy to the behavior of Ba^+ in the visible region. No evidence appears on these plates for the existence of Cs in the spot spectrum, a confirmation of Miss Moore's conclusion that this element is not observable in the sun. Ca, Fe, Cr and Ni exhibit either weakening, strengthening or no change, just as in the visible part of the spectrum, in accordance with the excitation potentials.

One of the most striking features of the infra-red spot spectrum is the enormous enhancement of many Ti lines. These will probably be found, on further comparison, to strengthen as much as any metallic lines in other spectral regions, e.g., the red lines of V.

A number of new lines, not thus far seen in the spectrum of the solar disc, are now found in the spot. These are apparently of atomic origin; no new bands have been observed.

It is well known that the chief obstacle in the study of the sunspot spectrum in the ultra-violet is the atmospheric and instrumental scattering of light from the disc. Although the Hale Solar Laboratory is located at an altitude of only about 700 feet above sea-level the new apparatus described above has proved distinctly useful for the observation of the ultra-violet region of the solar spectrum. Under observing conditions plainly below the best, a few spectrograms of sunspots have been made on which numerous effects can be seen as far as λ 3100. These make it clear that the spectrum of ultra-violet light scattered from the solar disc does not completely mask that of the umbra itself, though without doubt the spot spectrum is con-

taminated. Exposures five or six times as long as for the disc are required to attain the same photographic density in the spot.

The ultra-violet plates thus far obtained show many interesting effects. Band lines identified with the molecules OH and NH are stronger in the spot than in the disc, but the strong CN bands show no change. Large numbers of metallic lines are intensified in the spot and a few are perceptibly weakened, while many show no definite change from their appearance in the disc. The weakened lines thus far noted in the ultra-violet are those of ionized elements, e.g., Ni^+ . Among the most noticeably strengthened lines are low level multiplets of Ti and V, as would be expected. The behavior of a few heavy elements is particularly interesting, especially Ag, Cd, Rh, Ru and Pd, whose ultimate or penultimate lines are undoubtedly strengthened in the spot. No evidence is found in either disc or spot for the presence of Tl, whose ultimate line is at λ 3775.

Much detail awaits examination on the plates already made, and, with some further instrumental improvements under way, new sunspots of large diameter will be eagerly awaited.

¹ *Atomic Lines in the Sunspot Spectrum*. Princeton (1933). Mt. Wilson Contr. No. 446, *Astrophys. Journ.*, 75, 222 (1932).

SECOND NOTE ON A METAGALACTIC DENSITY GRADIENT

BY HARLOW SHAPLEY

HARVARD COLLEGE OBSERVATORY

Communicated November 15, 1938

1. An earlier note¹ on large non-uniformities in the metagalaxy presented evidence that in a zone thirty degrees wide, extending for 125 degrees across the south galactic polar cap, the density of population of eighteenth magnitude galaxies varies conspicuously. In addition to local irregularities of two or three hundred per cent from one area of nine square degrees to the next, there is a general tendency toward increasing population from a region in Aquarius at galactic latitude -60° , longitude 45° , to a region in Pictor, at galactic latitude -35° , longitude 225° , some two hundred million light years distant. It appears beyond doubt that this gradient is an important structural feature of the metagalaxy, and cannot be attributed to variations in space absorption.

The existence of inequalities in nebular density within limited areas of a few tens of degrees is easily shown and quantitatively measured on the large-field Harvard plates. There is need for caution, however, in attempting to obtain quantitative measures of the long-range gradient, referred to

above, which extends over a considerable interval in both right ascension and declination. One uncertainty in making a census of faint galaxies arises from uncertainties in the stellar photographic standards upon which we must base our reduction of nebular counts to a common distance and magnitude limit.

For the tabular and graphical results of the earlier note, the nebular counts were reduced in the best way that is at present available. They were brought to a common stellar magnitude limit by means of the well-known tables of Seares and van Rhijn, which give the relation between star numbers, apparent photographic magnitudes and galactic coördinates.

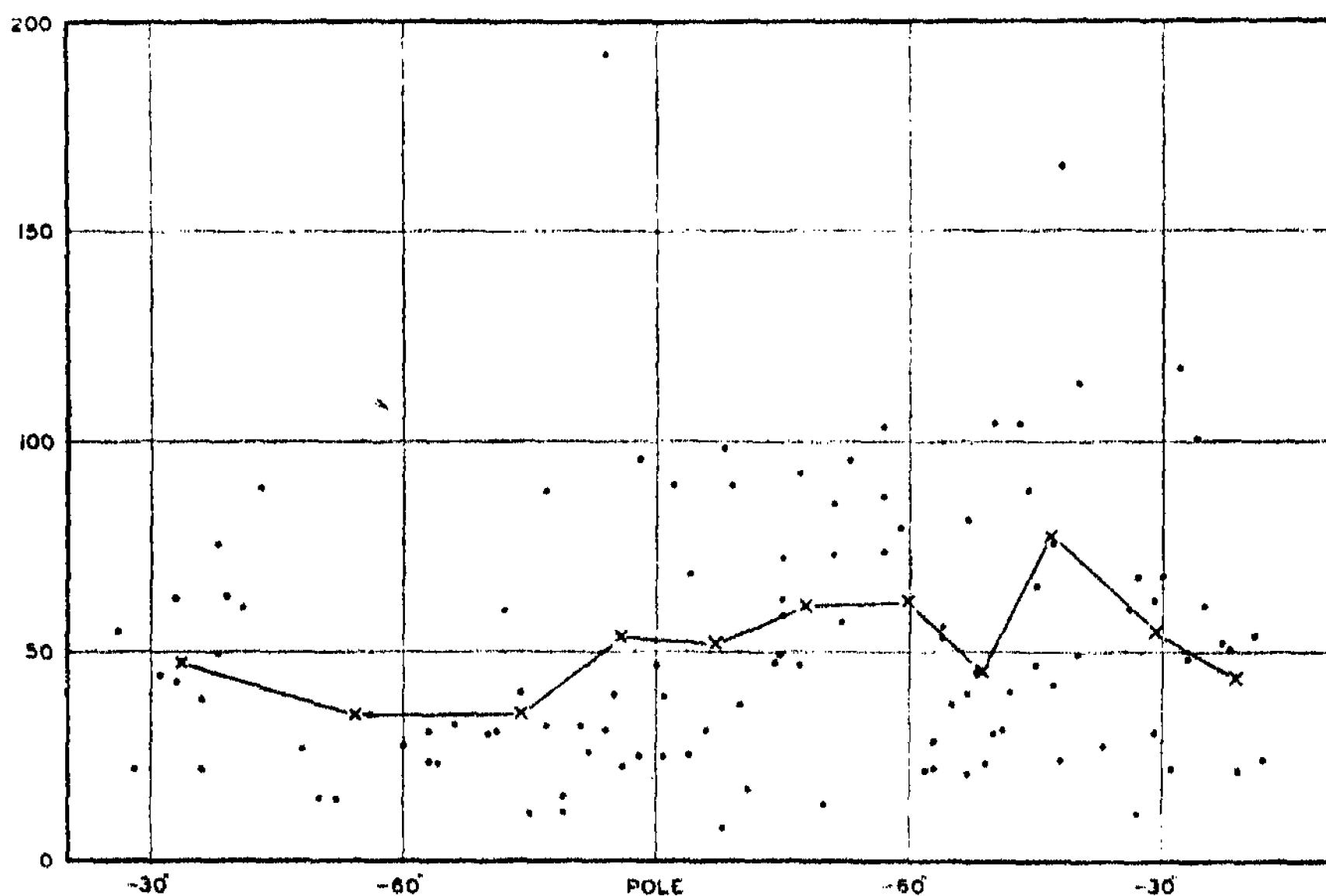


FIGURE 1

Distribution of galaxies in zone across the South Galactic Polar Cap. Ordinates are numbers per square degree; abscissae, distance in degrees along the zone.

The procedure has been pretty well standardized. On the three-hour Bruce plates the magnitude sequences, used in the determination of the magnitude limits for galaxies, have been established by the star-count method, which has proved to be highly satisfactory in northern declinations (north of $\delta = -20^\circ$) where the basic Selected Area sequences have been extensively studied by Seares and his collaborators at Mount Wilson. In southern declinations, however, the basic Selected Area sequences are as yet insecurely established, and the tables are therefore less dependable. There is consequently some risk in the reduction of the nebular counts to a common magnitude limit by this method.

That such procedure is at times precarious is indicated by the fact that groups of Bruce plates taken with identical exposure time, zenith distance and general observing conditions occasionally show a variation in plate limit apparently dependent on galactic coördinates but actually resulting from peculiarities of the van Rhijn tables.

2. To free the evidence for a metagalactic density gradient from the suspected errors of the existing star-count tables, we need only to work with the *unreduced* numbers of galaxies on the individual plates. The following tabulation gives the material in summarized form. Each entry represents ten plates, covering ninety square degrees. In this study of the long-range inequality, the local irregularities are, of course, largely smoothed out by the grouping. Figure 1 again shows that, independently of the stellar magnitude system, there is a strong metagalactic gradient.

TABLE 1
THE DISTRIBUTION OF FIFTY THOUSAND GALAXIES

LONGITUDE	GALACTIC LATITUDE	NUMBER PER SQUARE DEGREE	NUMBER PER SQUARE DEGREE, REDUCED
45	33.8	47.57	37.28
45	54.0	34.65	30.57
45	74.0	35.32	32.68
45	85.9	53.78	32.87
225	82.9	52.19	33.03
225	72.2	61.13	43.41
225	60.1	62.09	65.41
225	51.4	45.59	60.03
225	43.3	77.48	109.44
225	30.9	54.82	75.29
225	21.3*	43.85	66.74

* Six plates are involved in this mean, covering fifty-four square degrees.

The difference in slope between the curve in figure 1 and that given in the earlier note is both conspicuous and important. It affords direct evidence that at the eighteenth magnitude the system of stellar magnitudes in the southern hemisphere—at least along the 45° , 225° longitude circle—is not dependable. Before satisfactory quantitative work on the magnitudes of faint galaxies south of $\delta = -20^\circ$ can be undertaken, there must be a general investigation of the magnitudes of southern Selected Areas, and a subsequent correction or replacement of the star-count tables.

In figure 2 the same material as that of figure 1 is represented, but each plate has been reduced to a common magnitude limit by means of the van Rhijn tables, but without employing the corrections for galactic longitude. That is, for each galactic latitude and apparent magnitude the mean value of the star numbers has been adopted, rather than the published different values for different galactic longitudes. We have thus made use of the star

counts on the photographic plates to indicate their effectiveness in recording galaxies, and have not assumed average equality for all plates. This procedure is intermediate between that used in the earlier communication (unqualified use of the van Rhijn tables) and the procedure above, in which no reduction whatever is made on the basis of star-counts. This intermediate procedure probably gives us the best picture available at the present time. Certainly it gives to the results from the individual plates (plotted as small points in figure 2) more significance than the corresponding points in figure 1, since the unreduced values must include the true

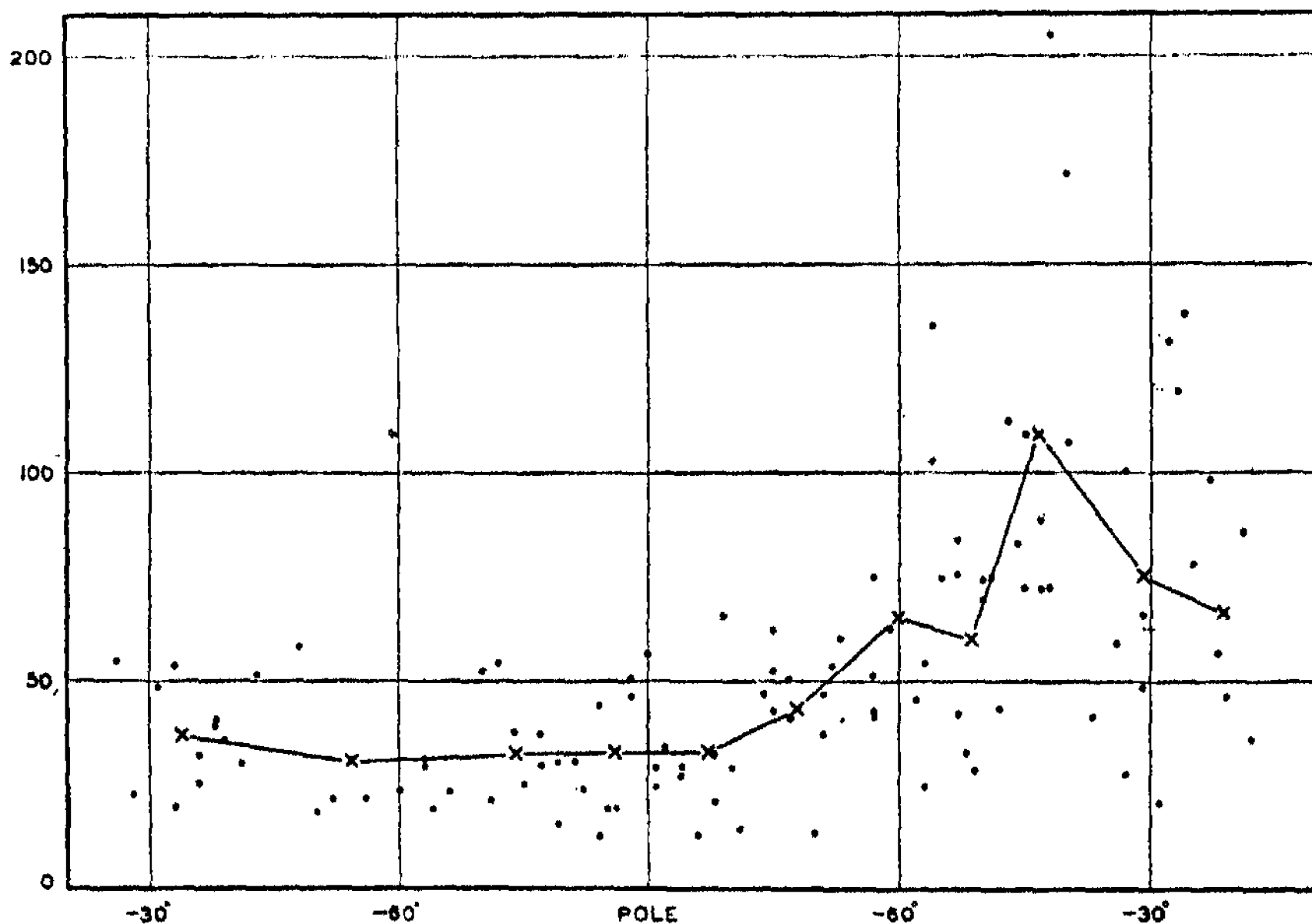


FIGURE 2

Same as figure 1, but the numbers have been reduced to a common magnitude limit.

inequalities in the penetrating power of the individual plates as well as the non-uniformity in the distribution of galaxies.

3. We might inquire as to other factors that could contribute illusory data on the metagalactic gradient. Since the results from the unreduced material summarized above depend on the assumption of average uniformity in plate effectiveness (for means of ten), we should consider the possible changes in emulsion speed, differences in plate quality, changes in transparency with the season and differences in zenith distance at mid-exposure. It is found on close examination that none of these factors contributes appreciably to the production of a spurious gradient. If anything, they have diminished the systematic inequality, as can be inferred by com-

paring figures 1 and 2 of this note. Space is not taken here to report on the details of examining these points, since the program of rectifying the star-count tables is now under way, and we should soon be able to reduce each southern plate directly and satisfactorily to a common magnitude limit and therefore make unnecessary the application of rather uncertain corrections for plate quality, zenith distance and seasonal effects.

In an earlier communication² the large inequalities in nebular densities over the south galactic polar cap were discussed. The question arises as to whether the deficiencies in the star-count tables may have contributed erroneous deductions in that study. It is easily seen that, in the mean, the inequalities are essentially the same whether or not the star-number tables are used. Thus we have for the eastern and western halves of the south galactic polar cap the number of galaxies per square degree:

	EAST	WEST
Reduced to 18 ^m .2	60.82	43.14
Unreduced	57.36	44.06

These results refer to the central nine square degrees of the eighty plates involved. For the surrounding sixteen square degrees on these plates, we have:

	EAST	WEST
Reduced to 17 ^m .87	40.07	27.67
Unreduced	37.52	25.10

In these comparisons, errors in the star-number tables depending on declination do not affect the results, since the intervals of declination involved are the same for both the western and the eastern sections of the polar cap.

¹ These PROCEEDINGS, 24, 282 (1938).

² *Ibid.*, 24, 148 (1938).

REFLECTIVITY AND COLOR OF METEORITES

BY FLETCHER G. WATSON

HARVARD COLLEGE OBSERVATORY

Communicated November 15, 1938

The principal data useful in establishing the physical characteristics of solid extra-terrestrial material, e.g., asteroids, are obtained from studies of spectral-reflectivity (color and albedo), phase effects and degree of polarization of the reflected light. For comparison with the extra-terrestrial bodies the earth-bound investigator has only two types of material: the various telluric stuffs and meteorites. Although the former are the more plentiful, we might expect the latter, of extra-terrestrial origin, to be more comparable to the interplanetary bodies. Reports of only two experiments upon the spectral-reflectivity of meteorites have been found. The first¹ of these is the bare statement that a highly polished and specularly reflecting surface of "Atacama"* gave sixty-four per cent in the green, fifty-nine per cent in the yellow and fifty-eight per cent in the red; thus suggesting that the light is made bluer upon reflection. The second study² revealed through photographic spectral-photometry that between λ 4000 and λ 6500 a fresh cleavage surface of Saratov (gray spherical chondrite, fell September 11, 1918, between 4 and 6 P. M.) reflected approximately twenty-five per cent of the incident light with a gray or uniform reflecting power. To make possible extensive comparisons between the extra-terrestrial bodies and the materials which can be examined in the laboratory, more data of this nature, covering a wide variety of meteoritic materials, are greatly to be desired.

The self-recording spectral-photometer in the Color Measurement Laboratory of the Massachusetts Institute of Technology is well adapted to the study of meteoritic reflectivity. Two restrictions are placed by the instrument upon the samples studied: (1) They should be nearly flat, and (2) they should have a surface at least one inch square. To these instrumental restrictions a third is necessarily added: The surface should not show weathering or discoloration from rust. Professor Charles Palache kindly permitted the use of the collection of meteorites at the Harvard University Museum. Many stone specimens in this collection were found to approximate the requirements, and seven were selected as representative of the various types and shades of meteorites. In six cases the surface examined was a broken surface approximately flat; the other sample, Mighei, had a roughly sawn surface. Such surfaces are necessary as we

* The nomenclature of Prior's Catalogue 1923, 1927, has been used throughout. "Atacama" is not a recognized identification, but is a synonym for two metallic meteorites from the Desert of Atacama: Imilac, pallasite; and Copiapo, brecciated octahedrite with silicate inclusions. It seems probable that Copiapo was the mass investigated.

expect the extra-terrestrial bodies to have rough surfaces comparable to those obtained upon fracture. Although the majority of iron meteorites had polished surfaces, a sample of Canyon Diablo was found with a rela-

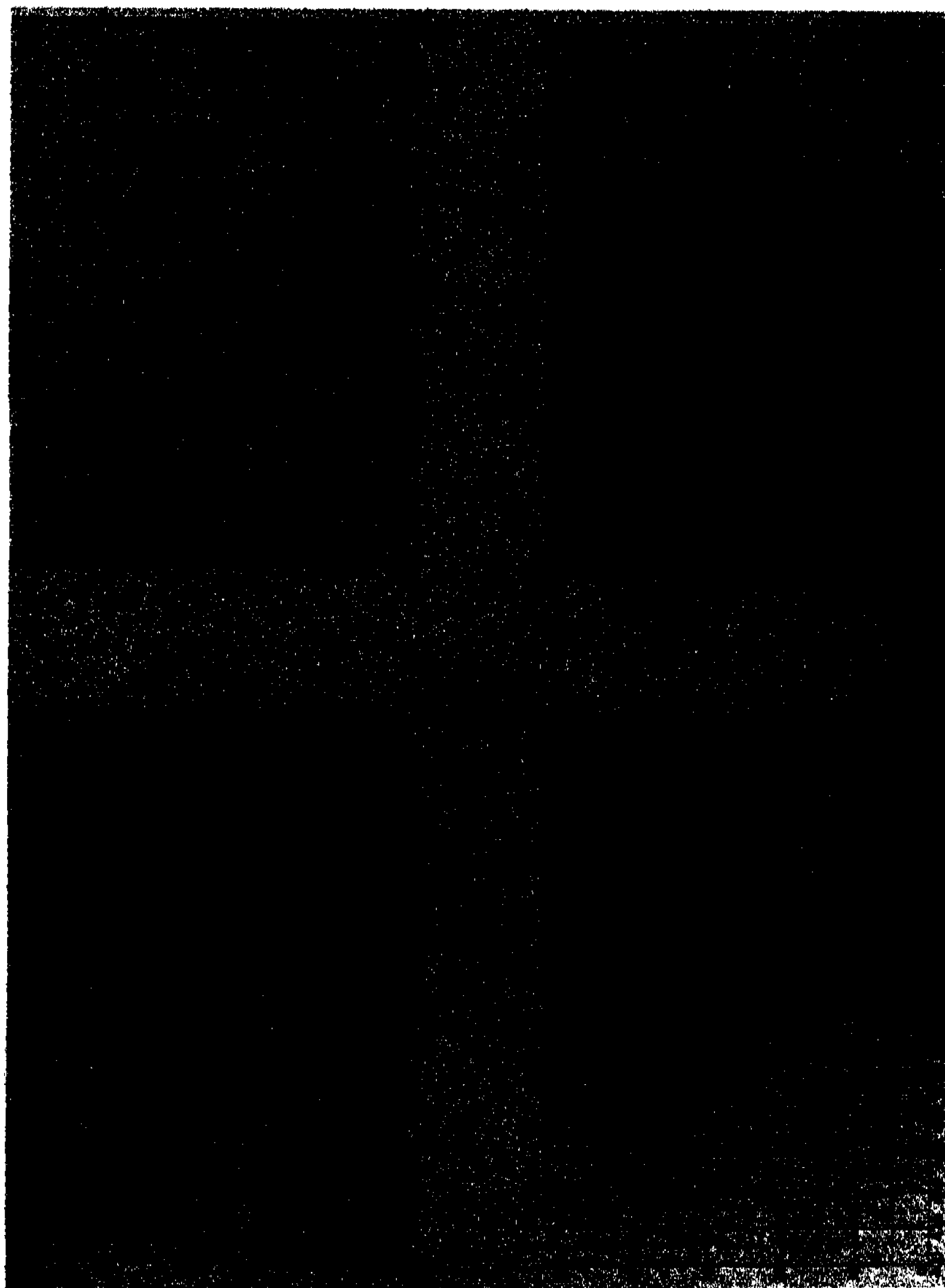


FIGURE 1

Spectral-reflectance curves for meteorites.

Allegan	Drake Creek
Wacanda	Olmedilla

Abscissae are wave-lengths in millimicrons; ordinates, percentage reflectance.

tively rough surface formed by deep etching after superficial polishing. It was examined to determine whether or not metallic and stony materials could be distinguished on the basis of color.

The spectral-reflectivities of these specimens are shown in figures 1 and 2. The descriptions of the surfaces examined are given in table 1. Comparison was made with a magnesium-oxide surface whose reflectance was

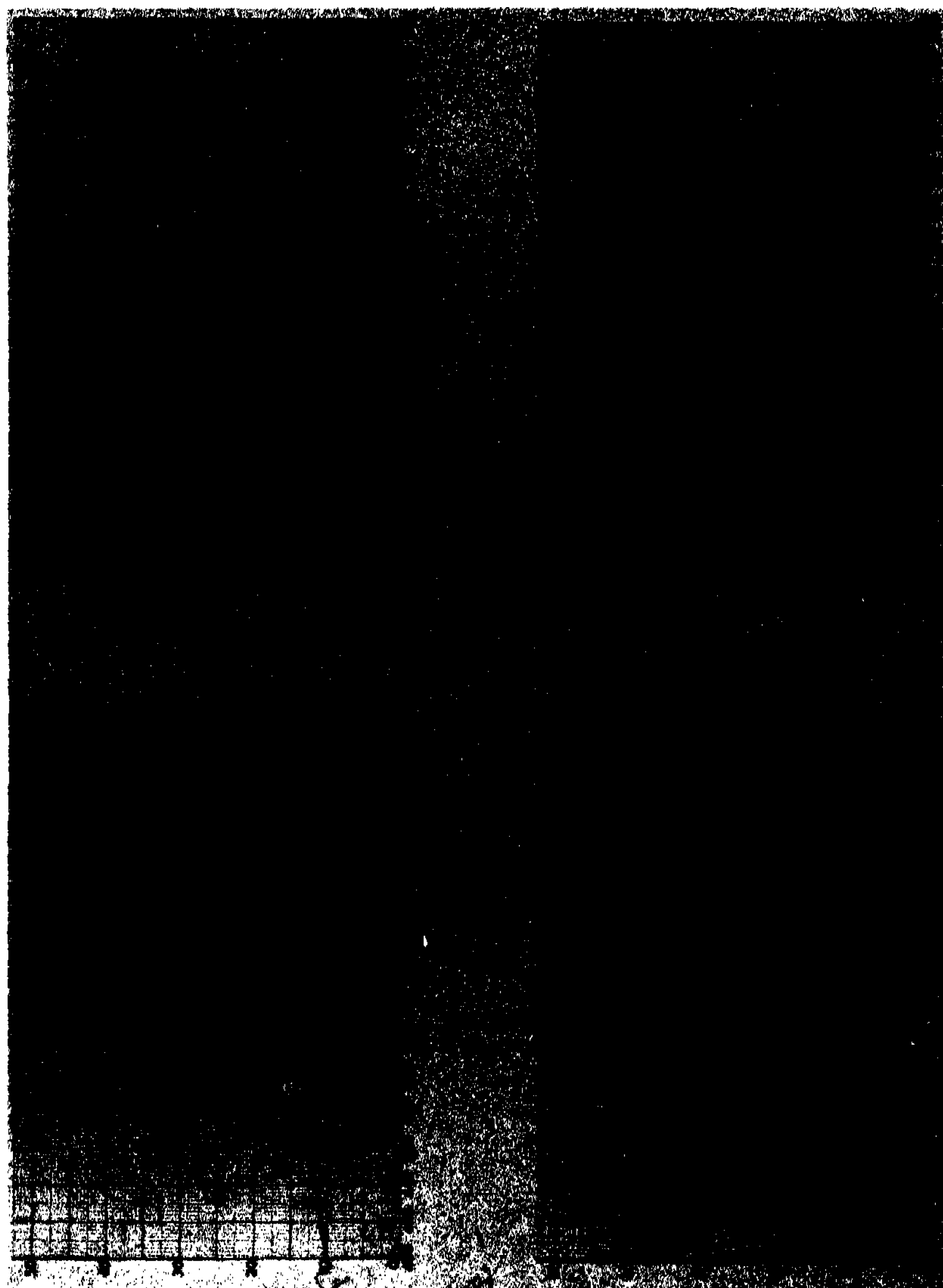


FIGURE 2
Spectral-reflectance curves for meteorites.
Parnallee Canyon Diablo
Farmington Mighei
Coördinates as in Figure 1.

checked against the national standard and found to be 0.98 of the incident light. The reflectance is accurate to 0.3 per cent and the scale of wavelengths to one Angstrom unit. The step-like variations at the blue end of

the curve for Waconda, the first specimen examined, resulted from a small instrumental time-lag which later disappeared. Other meteorites may be roughly placed on the reflectance scale by comparison with figure 3. The shades used are those which describe the surfaces examined and are comparable to the usual scale of shades employed in the description of mete-

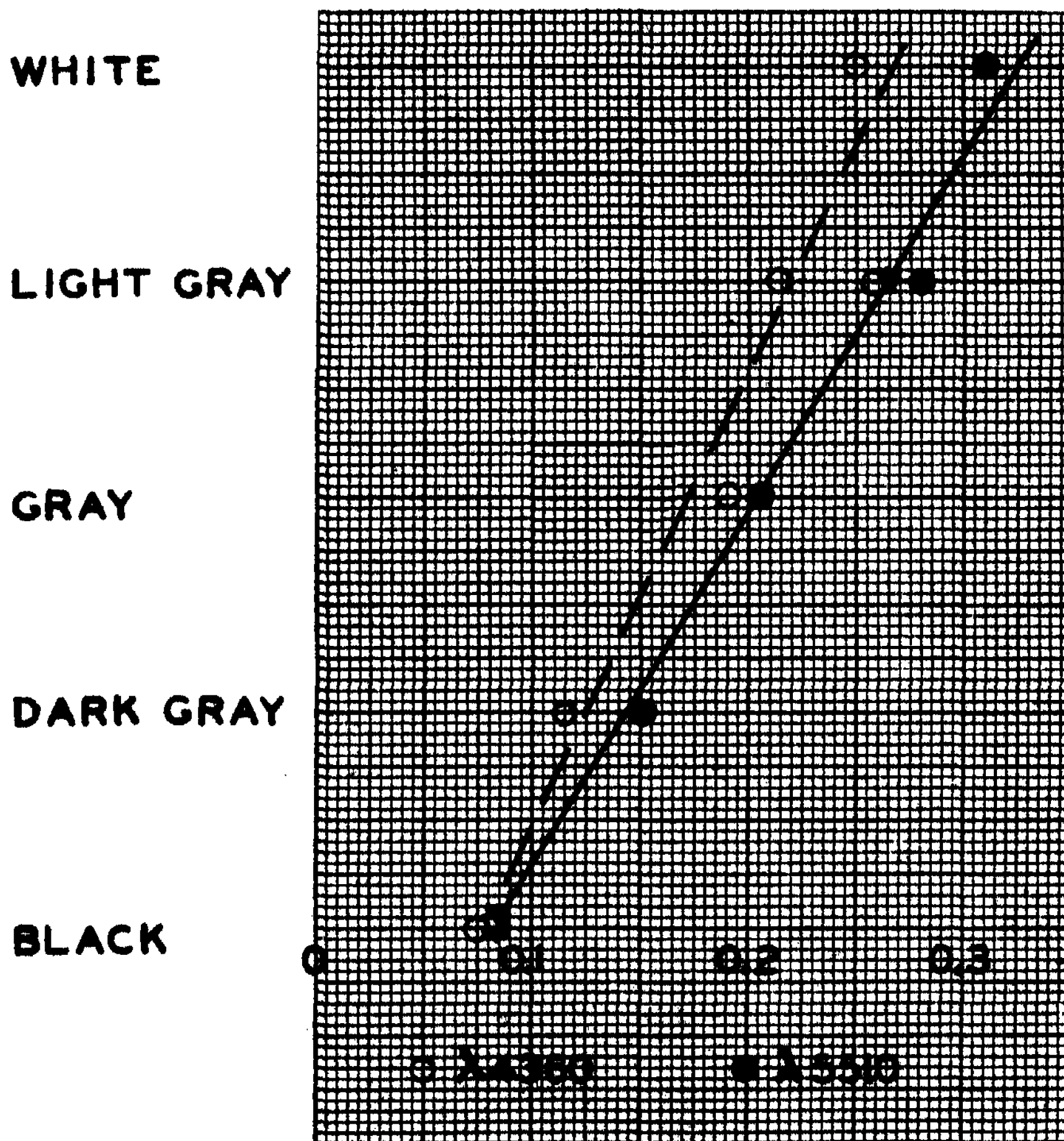


FIGURE 3

Reflectance values for meteorites of different shades.

orites. The flatness of the reflectance curves for the rust-free specimens shows that they are essentially "gray-bodies" over the spectral region in which sunlight is most intense. Thus the assumptions involved in the calculation of black- or gray-body temperatures for these masses in space are justified.

Color indices may be derived from the reflectance curves. The effective wave-lengths customarily used in the establishment of such indices are: photographic λ 4360, photovisual λ 5510 and red λ 6320. For these wave-lengths the reflectance values are transformed into color and red indices as given in table 2. Uncertainties in the intensities will not influence any indices except those of Farmington, which may be uncertain by 0.01 or 0.02 magnitudes. The three samples most colored are those most stained by rust and therefore they cannot be considered as representative. The other stone meteorites have color indices of approximately $+0.10$ magnitude. The color and reflectance values for the sample of Canyon Diablo suggest that on the basis of such data metallic and stony materials cannot be distinguished.

TABLE 1

DESCRIPTION OF SPECIMENS EXAMINED
STONE METEORITES

Allegan	Michigan (Specimen 480a), fell July 10, 1890, 8 A. M. Oransite chondrite, very friable. Surface rough, many chondri, no rust stains, considered as light gray.
Drake Creek	Nashville, Tennessee (Specimen 105b) fell May 9, 1827, 4 P. M. Veined white hypersthene-chondrite, some rust stains, considered as white.
Farmington	Kansas (Specimen 439c), fell June 25, 1890, 1 P. M. Black hypersthene-chondrite, few metallic inclusions, no rust stains, considered as black.
Mighei	Ukraine (Specimen 552), fell June 18, 1889, 8:30 A. M. Carbonaceous chondrite, sawn surface examined, some light chondri, no rust stains, considered as black.
Olmedilla de Alarcon	Spain (Specimen 580), fell February 26, 1929. Gray brecciated chondrite, no rust stains, considered as gray.
Parnallee	Madras, India (Specimen 233e), fell February 28, 1857, noon. Veined gray hypersthene-chondrite, many rust stains, considered as dark gray.
Waconda	Kansas (Specimen 344f), found 1873. Brecciated spherical hypersthene-chondrite, a few rust stains, considered as light gray.
IRON METEORITE	
Canyon Diablo	Arizona (Specimen 440e), surface deeply etched after slight polish, little specular reflection.

To evaluate properly the data given by the reflectance curves we must partially describe the measuring machine. The photosensitive surface is essentially a sphere; the sample is placed over a small opening in it opposite to the incoming beam of parallel light. At various wave-lengths the intensity of reflected light is compared photo-electrically with that from a magnesium-oxide surface identically situated in a second chamber. The reflectance is the integration of all light reflected from a "flat" surface over

the hemisphere of phase angle less than ninety degrees. In the terminology of the Bond albedo used in astronomy³ the reflectance is comparable to total albedo rather than to the "*p*-term," or geometrical albedo. Because of the rough, irregular surfaces of the meteorites examined it is likely that their "*q*-terms" are less than unity; 0.7 has been adopted provisionally, with recognition that the average asteroidal value is 0.55 (Russell, *op. cit.*). On the basis of this assumption geometrical albedoes, "*p*-terms," are given in table 2.

TABLE 2
REFLECTIVITY AND COLOR INDICES OF METEORITES

NAME	I_p	I_b	I_r	I_b/I_p	I_r/I_p	C. I.	R. I.	" p_{vis} "*
Allegan	0.257	0.280	0.284	1.09	1.11	+0 ^m .09	+0 ^m .11	0.40
Drake Creek	0.250	0.310	0.323	1.24	1.30	0.23	0.29	0.44
Farmington	0.075	0.085	0.087	1.13	1.16	0.13	0.16	0.12
Mighei	0.079	0.085	0.084	1.08	1.06	0.08	0.06	0.12
Olmedilla	0.190	0.205	0.205	1.08	1.08	0.08	0.08	0.29
Parnallee	0.115	0.151	0.163	1.31	1.42	0.29	0.38	0.22
Waconda	0.213	0.262	0.273	1.23	1.28	0.22	0.27	0.32
Canyon Diablo	0.143	0.172	0.186	1.19	1.28	0.19	0.27	0.25

* Computed on the assumption that $I_p = 0.7 p_{vis}$.

Summary.—From the spectral-reflectivity curves for meteorites, seven stony and one metallic specimen, it is concluded that (1) meteorites are essentially gray-bodies with color indices less than +0.2 magnitude; (2) their geometrical albedoes probably range from 0.1 to 0.5; (3) metallic and stony materials are probably indistinguishable on the basis of color data.

¹ Schneiderhöhn and Ramdohr, *Lehrbuch der Ermikroskopie*, 2, 89 (1931).

² E. Krinov, *Russ. Astr. Journ.*, 14, 356 (1937).

³ Russell, *Ap. Jour.*, 43, 101, 173 (1916).

ON THE DUPLEXITY THEORY OF VISUAL RESPONSE IN VERTEBRATES. II

By W. J. CROZIER AND E. WOLF

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated November 15, 1938

I.—The duplexity theory of visual performance in vertebrates is to the effect that retinal rods and their associated visual purple are connected with scotopic vision, indistinct and colorless, cones with photopic vision, distinctness and color. The predominance (partial or exclusive) of cones in the retinas of diurnal and of rods in nocturnal animals, and the presence of both in animals characteristically active both by day and night, is a very general rule.¹

By use of the technique based upon response to visual flicker it has been found that the majority of vertebrates give a relationship between flash-frequency F and flash-intensity I critical for recognition of flicker² which includes 2 (in one case³ 3) segments.

For the diurnal turtle *Pseudemys*, with purely cone retina, the $F - \log I_m$ curve occupies the general position of that for the "cone" segment of other vertebrates^{2,4} (with allowance for temperature); the curve is in this case a simple probability integral over its whole extent. The testing of nocturnal animals with purely rod retinas provides material critical for the evaluation of certain general propositions of the duplexity theory in its traditional form. Such an animal is found in the gecko *Sphaerodactylus*.⁵ The threshold response to flicker which is the basis for the measurements consists in a movement in the direction of the rotation of a striped cylinder;⁶ the relation of flicker-recognition to other forms of intensity discrimination⁷ indicates that the flicker-response contour is a delicate and precise test of general visual function.⁸

II.—The dissection of the duplex $F - \log I$ contour for most vertebrates can be made by use of the extrapolated probability integral which describes the upper or "cone" segment.^{2,9} If the lower segment is to be assigned to the activities of retinal rods, in keeping with the elementary form of the duplexity doctrine, then we should expect that for a purely rod-retina animal one might obtain an $F - \log I$ contour rising rather slowly to a comparatively low maximum, and with an inflection point at a comparatively low flash-intensity. It might also be found that beyond a certain maximum level of F the curve then declines,¹⁰ but there is evidence¹¹ that the falling off of the "rod" contribution to the duplex curve is more probably due to the inhibitory action of the "cone" effects.

The measurements made with *Sphaerodactylus* are collected in figure 1. The expansion of the iris⁶ of the gecko eye below a flash-intensity of ca.

0.0002 millilamberts causes the critical intensity to be mechanically lowered. This is proved by the effect of instilled pilocarpine, which constricts the pupil at low intensities, and by the measurements with iris expanded by atropine at higher intensities (see figure 1).

The curve drawn through the observed points is a probability integral. Its maximum F (26.51 for I_m ; 26.79 for F_m^{12}) is low by comparison with

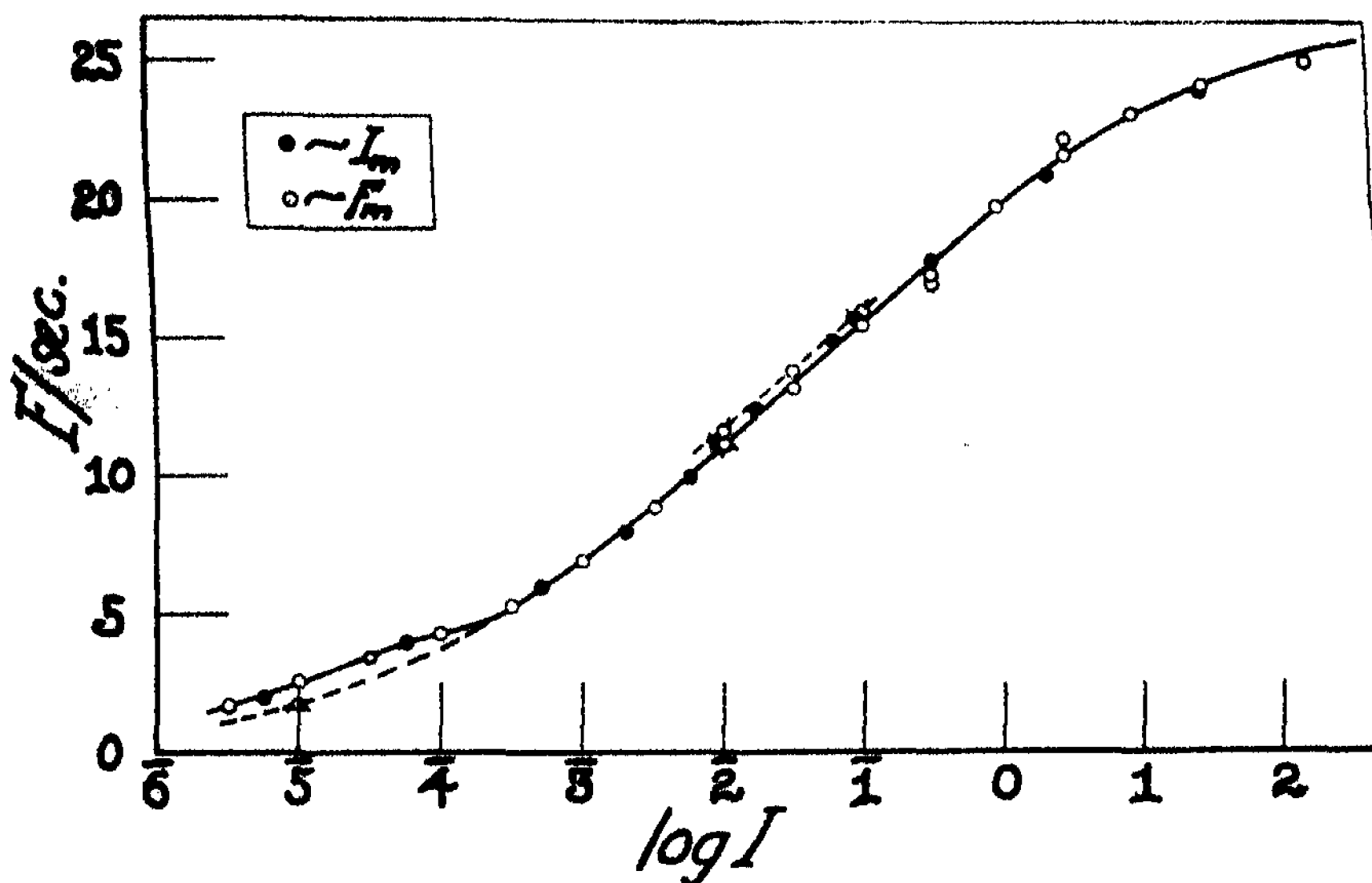


FIGURE 1

The flicker response contour for the gecko *Sphaerodactylus inaguae* Noble. Solid dots, $\log I_m$ at fixed flash-frequencies F ; open circlets, F_m at fixed flash-intensities; circlets with tags on the lower side, determinations after instillation of pilocarpine solution (constricting the pupil); circlets with tags on the upper side, after instillation of atropine solution (opening the pupil). The full line with the lower dashed extension is a computed probability integral, which in the absence of the effect at the lower end (as proved by the influence of the constricted pupil) due to the opening of the pupil below $\text{ca. } \log I = -4.3$, gives an excellent description of the data. Temperature, $26.7^\circ \pm 0.6^\circ$; flash-cycle with 50 per cent light time. At each point, 3 observations on each of 10 individuals; the data are not homogeneous, in the sense that the same 10 were not used for the different tests (hence the distinction⁶ between I_m and F_m is not apparent).

that for all other animal types tested (47 to 63, under the same general conditions). Its inflection point, however, is at a high intensity close to those for the "cone" sections of the curves of other vertebrates.

III.—The lowness of F_{max} could be a consequence of the small size of the eye, which should also tend to increase the value of $\log I$ at the inflection. The comparison between the curves for a purely cone-^{2,4} and purely rod-retina animal can be most clearly made by bringing them to

the same value of F_{max} . (100 per cent). This will not affect $\log I$ at inflection ($= \tau'$), and permits comparing the spread constants $\sigma'_{\log I}$ since the form of the function is in each case that of a probability integral F_{max} . is a specific property of the type of animal, but is not directly correlated with τ' or $\sigma'_{\log I}$.¹³ This comparison is made in figure 2.

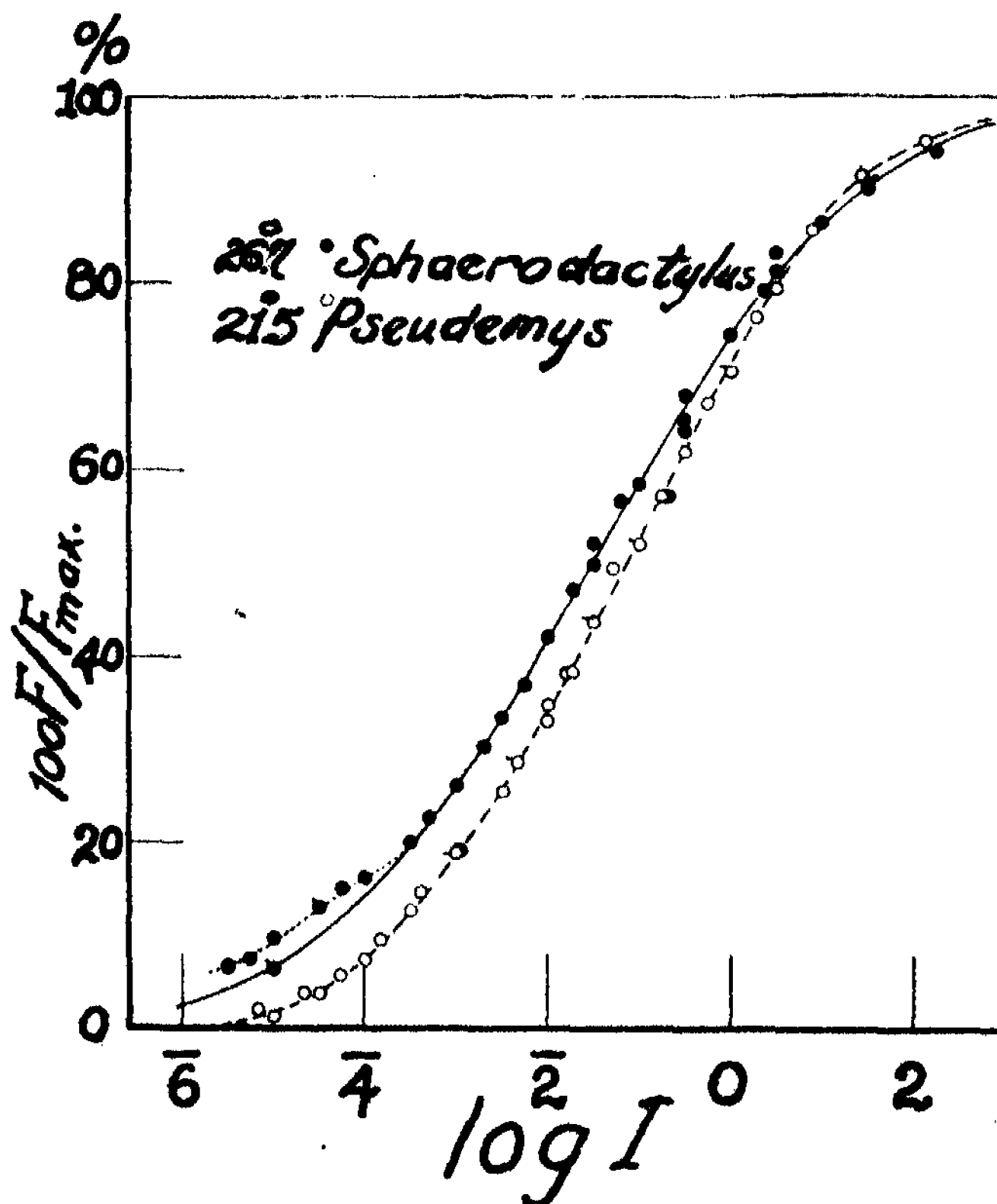


FIGURE 2

The flicker-response contours for *Sphaerodactylus* (gecko; rod retina) and *Pseudemys* (turtle; cone retina) compared by adjusting to a flash-frequency scale in which F_{max} . for each = 100 per cent. The curves drawn are the computed probability integrals. The slight difference is further reduced by correcting for the difference in temperature (gecko, 26.7°; turtle, 21.5°). The standard deviation ($\sigma'_{\log I}$) is a little greater for *Sphaerodactylus*.

The gecko covers the same range of intensities in its $F - \log I$ curve as does the turtle. The temperature was different in the two cases; applying the temperature characteristic found for the decrease of τ' with rise of temperature in *Pseudemys*,¹⁴ the values of τ' for gecko and the turtle differ

only by -0.08 log units. The values of $\sigma'_{\log I}$ differ by less than do those for different genera of fishes.¹³

IV.—These observations clearly give no support to the conception that a rod retina necessarily functions best at low illuminations, or less ably at higher illuminations than a cone retina,¹⁴ or that the excitabilities of rods are restricted to lower illuminations. They cast increased doubt upon the validity of specifically associating histological appearance and functional capacity. In the analysis of visual responses it is to be recognized that the occurrence of two component populations of effects in the constitution of the response contour signifies merely that these must be separately considered on the basis of the quantitative descriptions of their properties.² There need not necessarily be involved any direct correlation of these sets of properties with histological differentiations of the peripheral receptive field.

A fuller account of the experiments will shortly be published elsewhere.

¹ Scharrer, E., *Zeits. vergl. Physiol.*, 11, 104 (1929); Verrier, M.-L., *Bull. Biol. Fr. Belg.*, 71, 238 (1937).

² *Proc. Nat. Acad. Sci.*, 24, 125 (1938).

³ *Jour. Exptl. Zool.* (in press) [*Triturus*].

⁴ *Jour. Gen. Physiol.* (in press) [*Pseudemys*].

⁵ We are greatly indebted to Mr. R. A. McLean for a supply of these geckos collected at Matthew Town, Great Inagua, Bahama Islands; and to Dr. Thomas Barbour, Director of the Museum of Comparative Zoölogy, for his identification of them as *Sphaerodactylus inaguae* Noble.

⁶ *Jour. Gen. Physiol.*, 19, 495 (1935-1936); 20, 211, 393, 411 (1936-1937).

⁷ *Ibid.*, 19, 503 (1935-1936); 20, 393 (1936-1937); 21, 313 (1937-1938).

⁸ On the gecko retina, see: Rochon-Duvigneaud, A., *Ann. d'oculist.*, Nov., 35 (1917); Detwiler, S. R., *J. Comp. Neurol.*, 36, 125 (1923).

⁹ *Jour. Gen. Physiol.*, 20, 411 (1936-1937); 21, 17 (1937-1938); etc.

¹⁰ *Ibid.*, 20, 411 (1936-1937).

¹¹ *Ibid.* (in press) [Related fishes, II] (1938-1939).

¹² *Ibid.*, 20, 211, 363 (1936-1937).

¹³ *Proc. Nat. Acad. Sci.*, 23, 516 (1937); 24, 542-545 (1938); *Jour. Gen. Physiol.*, 21, 17 (1937-1938); (in press).

¹⁴ *Proc. Nat. Acad. Sci.*, 24, 216 (1938).

¹⁵ The relation of these findings to the hypothesis advanced by Walls that the nocturnal geckos are descended from ancestors with cone-retinas (Walls, G. L., *Amer. J. Ophthalmol.*, 17, 892 (1934)) is of course entirely a matter for speculation.

SPECIFIC CONSTANTS FOR VISUAL EXCITATION. III

BY W. J. CROZIER AND E. WOLF

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated November 15, 1938

I.—Curves are obtainable in a precisely reproducible way which define the dependence of mean flash-intensity I_m critical for response to visual flicker upon the flash-frequency F .¹ For various animals these curves are of the same fundamental form, and are accurately described by a probability integral² ($F - \log I_m$). The properties of the three parameters of this formulation ($\sigma'_{\log I}$; abscissa of inflection, τ' ; and maximum, $F_{max.}$), as revealed by their respective relations to temperature, retinal area and proportion of light-time in the flash cycle, are specifically consistent with this interpretation. The fundamental form is modified in most arthropods as a result of the convexity of the optic surface.⁴ With most vertebrates it is complicated by the existence of two sets of elements of neural effects, having different mean $\log I$ thresholds,⁵ so that the $F - \log I$ curve is then typically a complex of two overlapping sigmoid sections (which can be analytically separated⁶).

The specific differences between the $F - \log I$ curves for different kinds of animals gives opportunity to make a significant test of the invariant character of the parameters of the probability function. In forms with which adequate tests have been made thus far (turtle, sunfish, nymph of dragonfly) this specific invariance of $F_{max.}$ and shape constant $\sigma'_{\log I}$ under conditions producing change of τ' , and the invariance of $\sigma'_{\log I}$ under conditions changing both τ' and $F_{max.}$ are striking evidence³ for the propriety of the probability formulation. This is especially the case since in each of these instances the particular modification found is called for by the statistical conception of the basis for the expression of sensory effect.⁷

II.—The proof that simple constitutionally determined properties of the organism govern the expression of this sort of dynamical invariance can be obtained (but in a given test is not necessarily to be given in an obvious way) by means of genetic experiments.⁸ For this purpose fresh water teleosts provide exceptional material, since they permit intergeneric cross breeding. Thus the $F - \log I_m$ curves for *Xiphophorus helleri* (X., swordtail) and *Platypocoeilius maculatus* (P., platy) are quite different,^{8,9} hybrid descendants of their cross breeding (H') show definite inheritance of certain characteristics (shape constants) of the $F - \log I_m$ curve from one or the other parent.^{8,9} These tests also demonstrated that the two populations of excitation-effects ("rod" and "cone") could be genetically separated; so likewise that the mechanisms determining τ' and $F_{max.}$ can be genetically influenced in independent ways.

III.—The importance of such facts in furthering the establishment of methods for the recognition of measures of biologically significant invariant properties led to a repetition of our earlier experiment.⁸ First generation hybrids were obtained (H'') of two other species of the genera previously used, *X. montezuma* and *P. variatus*.

Adequate comparisons¹⁰ showed that within each genus the flicker-response performance of several types each of genera *X.* and *P.* gave essentially the same $F - \log I_m$ curve—even in the case of albino *X. helleri*.¹¹ Figure 1 exhibits the curves for *X.*, *P.* and their F_1 hybrids (H''). Just as in the earlier (H') experiment⁸ the upper segment of H'' is of precisely the same shape as that for *X.* The maximum for H'' is slightly higher, but $\sigma'_{\log I}$ is identical (Fig. 2). As with H' , τ' is intermediate. For the lower

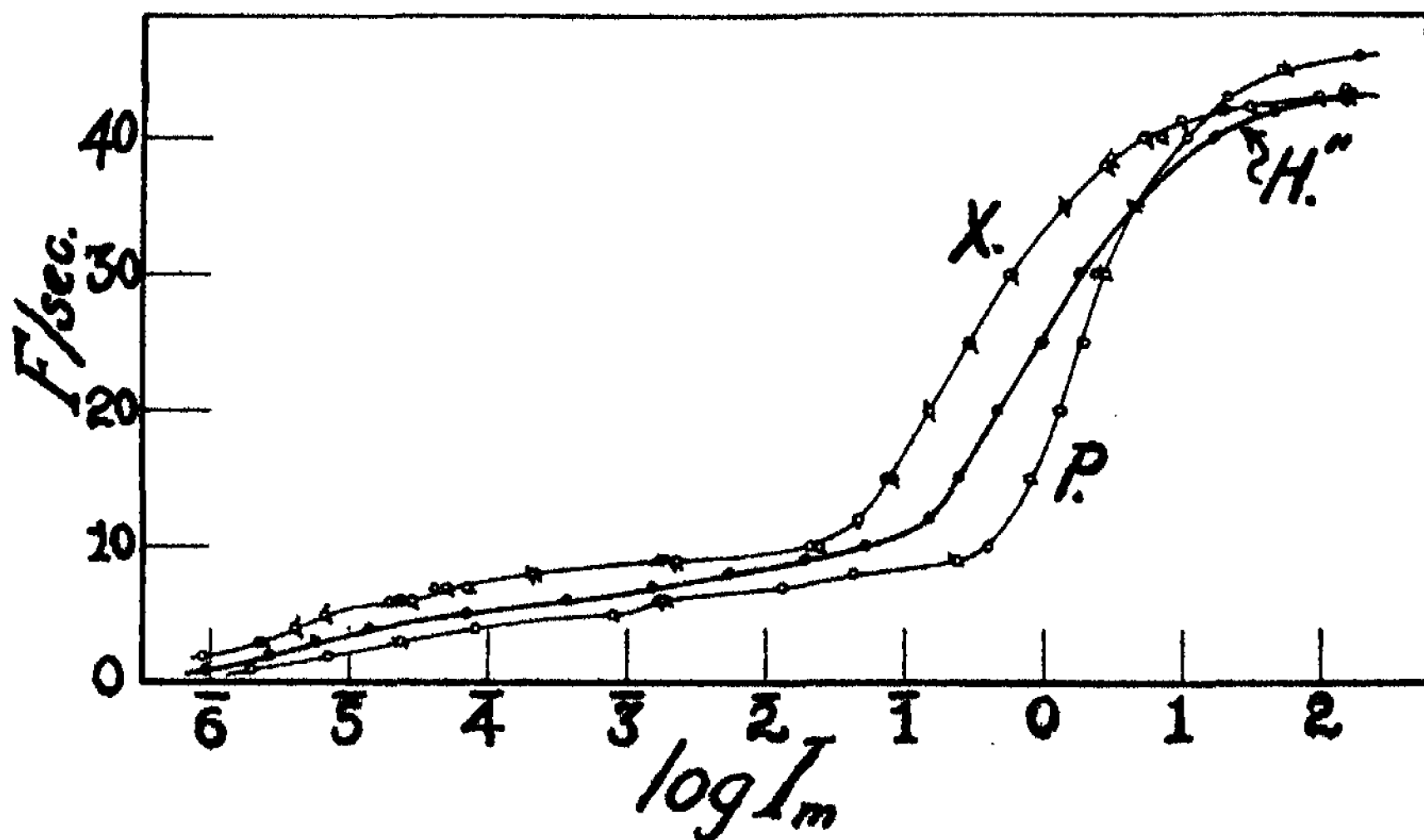


FIGURE 1

Flicker response contours for several types of *Xiphophorus* (*X.*), distinguished by different symbols, and including *X. helleri* and *X. montezuma*; for several varieties of *Platypoecilus* (*P.*), including *P. maculatus* and *P. variatus*; and for F_1 hybrids (H'') between *X. montezuma* and *P. variatus*. Temperature 21.5°, flicker-cycle with 50 per cent light time. For *X.* and *P.* the various series of plotted points are in different cases averages of 9 to 36 determinations each; for H'' each plotted point is the mean of 9 (3 individuals).

("rod") segment, H'' , $\sigma'_{\log I}$ corresponds to that for *P.*, not *X.* as with H' (cf.⁹), and F_{max} and τ' are intermediate (Figs. 1, 3).

IV.—The essential facts demonstrated by the analysis of the data in figure 1 agree with those found in our earlier experiment. The quantitative differences between F_{max} , τ' in H' and H'' may be due to the fact that the parent species were different in the two cases, or to the fact that H' was a (uniform) back-cross product. These differences are at the moment

interesting only because they supply an internal check on the precision of the method of observation.

The results show why it is essential to have contours of measurements of performance over a wide range of the governing variable before classification of individuals is possible, or before genetic interpretations can be unambiguous.¹² They also illustrate a method of testing the relevance of

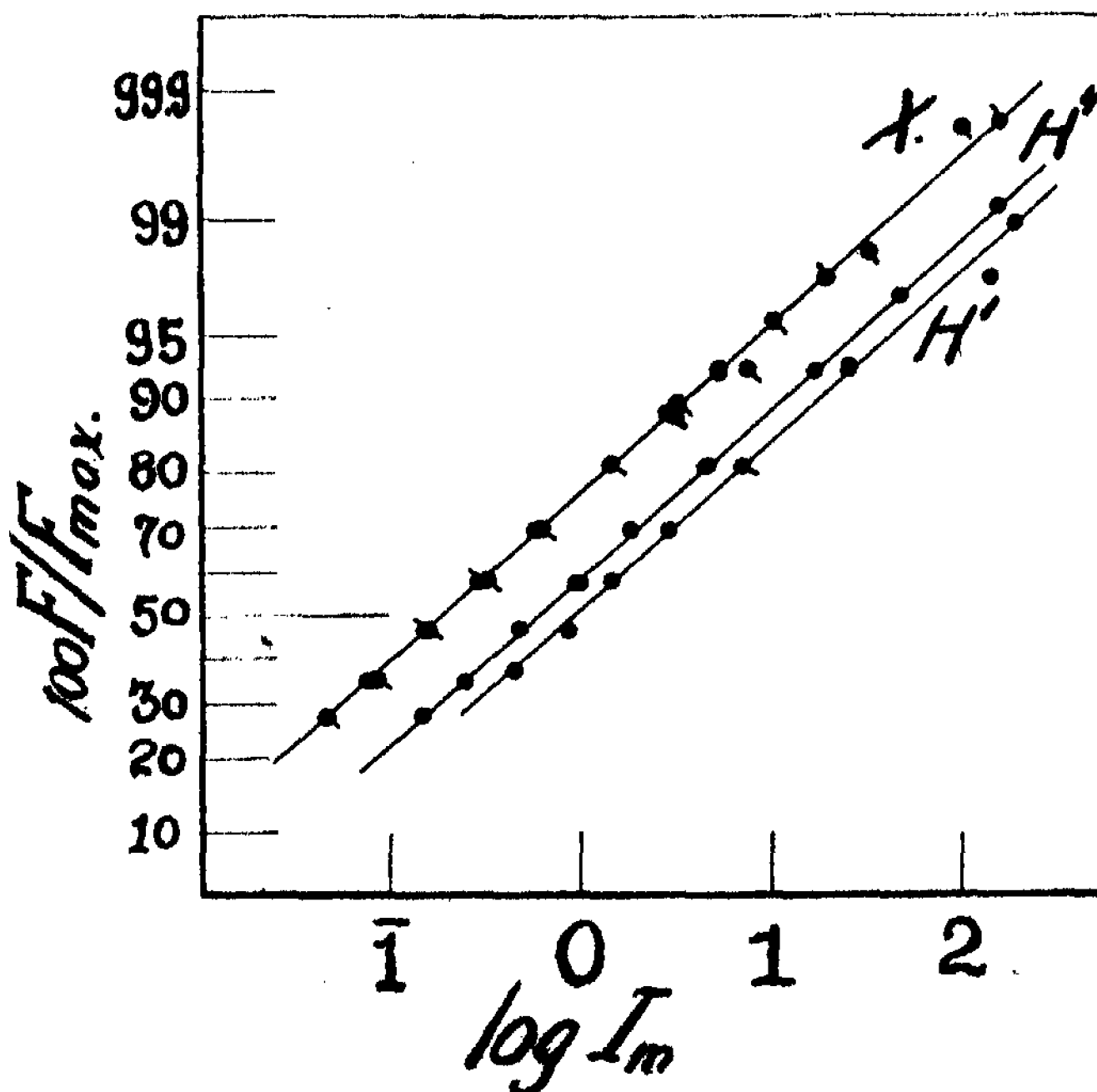


FIGURE 2

Data for *Xiphophorus* (X.) and for 2 types of its hybrids with *Platypoecelius* (H' and H''); only the upper or "cone" parts of the graphs in figure 1 are shown; H' is taken from a previous report.¹⁰ The three lines are drawn parallel on a probability grid; $\sigma'_{\log I}$ is consequently the same—that for *P.* is of course much smaller. The maxima are respectively F_{max} . equals for X. 43.05, H' 43.00, H'' 43.34.

parameters implicit in analytical formulations of biological properties. In the nature of the present data⁷ such formulations cannot be tested by curve fitting alone. The physical properties of the constants present must be suitable, and quantitatively appropriate.⁸ Genetic tests indicate independent properties of these parameters, as called for by experimental results with different variables in tests with individual organisms.⁸ They also show that determinate constitutional features of organization govern the exhibition of dynamical properties in biologically different animals. The properties

concerned in the invariant *form* of the flicker-response contour therefore must be properties arising from the fact that in different organic types physically different substrata produce dynamically similar results. These properties must therefore be essentially statistical.

What various kinds of animals have in common with respect to the invariant form of the flicker-contour is the possession of a large assemblage of (essentially variable) excitation elements. The parameters of their frequency distributions of effectiveness are fixed by the specific genetic composition, and must consequently depend upon the intrinsic properties of the physical substrata of these elements. This is entirely consistent with the directly determined control of the magnitudes of these parameters by temperature, area, and light-time cycle-fraction in different animals.⁸ The same argument applies to the demonstration of the existence of two populations of visual sensory effects in most vertebrates, correlated with the presence of both retinal rods and cones.⁹

A more detailed consideration of these data is in course of publication.¹⁰

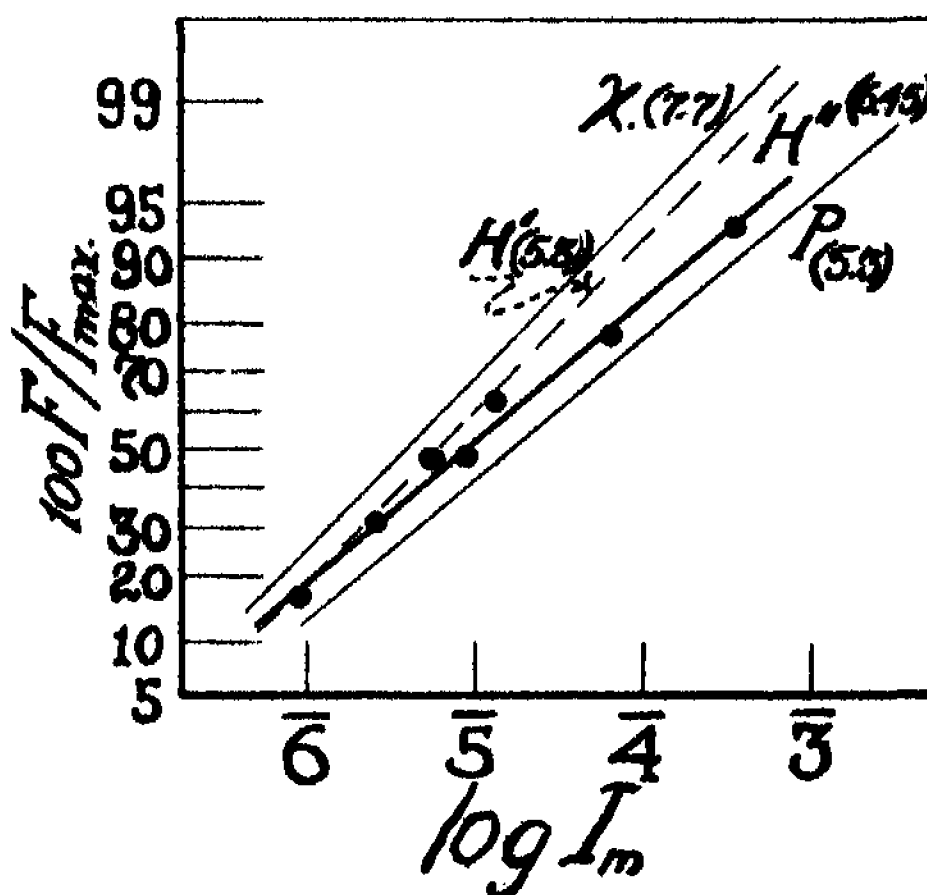


FIGURE 3

Data for $X.$, $P.$, H' and H in the lower or "rod" section of the flicker contour (cf. figure 1), are shown on a probability grid, with maxima as indicated giving best rectilinearity. The lines for $X.$, $P.$ and H' are taken from previous work.¹⁰

¹ *Proc. Nat. Acad. Sci.*, 23, 71, 516 (1937); 24, 125 (1938).

² *Ibid.*, 24, 125 (1938); *J. Gen. Physiol.* (in press).

³ *Jour. Gen. Physiol.*, 21, 223 (1937-1938); 20, 393, 411 (1936-1937); 21, 313, 463 (1937-1938) (in press); *Proc. Nat. Acad. Sci.*, 24, 216 (1938).

⁴ *Jour. Gen. Physiol.*, 21, 223 (1937-1938); (1938-1939) (in press).

⁵ *Ibid.*, 20, 411, etc., (1936-1937).

⁶ *Ibid.*, 20, 411 (1936-1937); 21, 313, etc. (1937-1938).

⁷ *Ibid.*, 19, 503 (1935-1936); *Proc. Nat. Acad. Sci.*, 22, 412 (1936); 23, 23, 130 (1937).

⁸ *Ibid.*, 23, 516 (1937).

⁹ *Jour. Gen. Physiol.*, 21, 17 (1937-1938).

¹⁰ *Ibid.* (in press).

¹¹ *Proc. Nat. Acad. Sci.*, 24, 221 (1938).

¹² *Jour. Gen. Physiol.*, 13, 57, 81, etc. (1929-1930).

THE EFFECTS OF CHEMICALS ON THE LETHAL MUTATION RATE IN *DROSOPHILA MELANOGASTER*

By L. W. LAW

SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY¹

Communicated November 14, 1938

Numerous attempts have been made to induce mutations artificially in *Drosophila* by means of chemical agents. The early procedures involved feeding of larvae chemical substances mixed with the food.²⁻⁶ Various poisons, acids and bases, and dyes all gave negative results. Later, Stadler⁷ and Medvedev⁸ showed that the salts of heavy metals, in conjunction with the application of x-rays, produced a significant increase in the mutation rate over that resulting from x-raying alone. This was in barley and *Drosophila*, respectively. In these cases, however, the substances probably only increased the effectiveness of absorption.

The Russian workers were the first to report indications of gene mutations, resulting from treatment of fertilized *Drosophila* eggs and larvae with various chemical substances. Lobašov and Smirnov⁹ reported an increase in the lethal mutation rate in *Drosophila*, the larvae of which were treated with NH_4OH . Lobašov¹⁰ also presented data indicating an increased lethal mutation rate due to treatment of larvae and pupae with acids, although Goldat and Beliaieva¹¹ could find no significant increase from egg treatment with HCl .

More recently a group of workers of the Bubnov Pedagogical Institute in Moscow reported increased mutation rates resulting from treatment of fertilized *Drosophila* eggs, chorion intact, with I_2 in KI , CuSO_4 and KMnO_4 .¹²⁻¹⁴ Preliminary data by Samjatina and Popowa¹⁵ and Kondakowa¹⁶ also indicated that iodine treatment produced mutations, although the lethal mutation rate (second chromosome) reported by the latter was extremely high for a relatively small number of chromosomes tested, $7.8 \pm 2.7\%$ for 121 chromosomes tested and 9.1 ± 1.7 for 285 chromosomes tested. More recently Lobašov¹⁷ found an increase in the mutation rate, lethal and visible, through injection of various strengths of NH_4OH and HCl into *Drosophila* pupae.

In plants Stubbe¹⁸ has reported the induction of mutations in *Antirrhinum* resulting from several specific chemical substances.

In some of the experiments reported above the strains of *Drosophila* used were not given, the incidences of lethal mutations were of doubtful significance, and there appeared as a result of the chemical treatment in some cases an abnormally high percentage of both lethal and visible mutations. Thus, in view of the fact that both negative and positive results are reported for the same substances, it seemed worth while, first, to use a

TABLE 1
THE EFFECTS OF VARIOUS CHEMICALS ON THE LETHAL MUTATION RATE IN THE X-CHROMOSOME OF THE OREGON-R RACE OF DROSOPHILA MELANOGASTER

EXP. NO.	CHEMICAL	TYPE	TREATMENT CONC.	TIME	NO. OF MALES TESTED	NO. OF X-CHROM. TESTED	NO. OF LETHALS	% LETHALS	LOCATION OF LETHALS	MUTATION RATE
A	CuSO ₄	Injection	0.1%	10 min.	6	471	3, 2*	1.06		
					5	480	3, 3*	1.25	I, II*, II*	
		Immersion of eggs	Conc.							
2A	CuSO ₄									
Total					11	951	6, 5*	1.16		1 in 86.4
B	KMnO ₄	Injection	0.025%	15 min.	4	405		
					4	447	1, 2*	0.66	I, II*, II*	
		Immersion of eggs	Conc.							
2B	KMnO ₄									
Total					8	852	1, 2*	0.35		1 in 284
C	I ₂ in KI	Injection	{ 0.038% I 0.075% KI	5 min.	6	386	2*	0.52		
					3	460	1*	0.22	I*	
		Immersion of eggs	50%							
2C	I ₂ in KI									
Total					9	846	3*	0.35		1 in 282
D	Colchicine	Injection	0.0013%	4 min.	4	421		
					4	305		
		Immersion of eggs	Conc.							
2D	Colchicine									
3D	Colchicine	Immersion of eggs	0.5%	15 min.	3	259		
Total					11	985		0 in 985
E	Ringers (Control)	Injection			7	507		0 in 507*

s = semi-lethal mutations. Those producing a sex ratio between 1:0.30 and 1:0. Only females not carrying ClB are considered, and only culture vials containing at least 50 flies were used. Lethals are located as I—between *sc* and *v*, and II—between *v* and *f*.

* Demerec²¹ found 2 lethals in 3049 X-chromosomes tested in this stock.

new technique for administering the chemicals, and second, to remove the chorion from fertilized eggs before treatment. The positive results published by the Bubnov authors were obtained through treatment of chorion-intact fertilized eggs.

In one set of experiments the chemical substances were injected into larvae by use of a micro-injection needle, following the method of Beadle and Ephrussi.¹⁹ In this manner from 0.5 to 1 cubic mm. of the substance may be injected into each larva. The gonads were thus bathed in the solution. For each chemical substance used the sub-lethal dosage was determined by injection of a series of concentrations into appropriate test larvae. In the second series of experiments the chorion was removed from fertilized eggs by gently stroking with a blunt glass needle. These were immersed in sub-lethal dosages of the chemicals. These dosages were determined by running a series of different concentrations over different time intervals. The various sub-lethal dosages employed in this study are listed in table 1.

I₂ in KI, CuSO₄, KMnO₄ and colchicine have been used in testing effects of the lethal mutation rate. The first three substances were used because of their reported influence of

TABLE 2 COMPARATIVE RESULTS OF THE LETHAL MUTATION RATE OBTAINED BY TREATMENT WITH VARIOUS CHEMICALS									
CHEMICAL USED	TYPE	TREATMENT CONC.	TIME	NO. OF SEPS. EXPS. TESTED	NO. OF X-CHROMES.	NUMBER LETHALS	% LETHALS	DIFF. % σ D %	AUTHOR
CuSO ₄	Eggs immersed	Conc. aqueous	40 min.	1	579	6	1.03	0.82 ± 0.29 ^r	Magrzhikovskaja, 1936
CuSO ₄	Eggs immersed	Conc. in alcohol of various strengths	5-40 min.	5	2826	27, 16*	1.52	1.31 ± 0.25	Magrzhikovskaja, 1936
CuSO ₄	See table 1			2	951	6, 5*	1.16	1.16 ± 0.48	This report
KMnO ₄	Eggs immersed	Conc. aqueous	5-80 min.	4	1101	7	0.63	0.39 ± 0.46	Naumenko, 1936
KMnO ₄	Eggs immersed	Conc. alcohol	5 min.	1	582	(24)*	(4.12)	1.39 ± 0.53	Naumenko, 1936
						9	1.55		
KMnO ₄	See table 1			2	852	1, 2*	0.35	0.35 ± 0.25	This report
I ₂ in KI	Eggs immersed	5% in 10% KI	15-20 min.	1	703	7	0.99	0.55 ± 0.49	Ssacharow, 1936
I ₂ in KI	See table 1			2	846	3*	0.35	0.35 ± 0.25	This report

^r All errors are standard errors.

* See text.

gene mutation and colchicine because of its apparent effect of mitotic processes.²⁰

The inbred Oregon-R stock has been used as test animals. Only the *X*-chromosome was studied, and lethals were detected by the usual *ClB* method:

- (1) *ClB*/*x-pl** female by + (treated) male
- (2) *F*₁ of *ClB*/+ female by *x-pl* male
- (3) Females from cultures indicating lethal or semi-lethal mutations were mated by *x-pl* males.

Flies for (1) were raised in 1/2 pint milk bottles, and for (2) and (3) in 25 × 95 mm. vials. The standard food of corn meal, molasses and agar, with brewers' yeast added, was used throughout.

Eggs were collected over a two-hour period at 25°C. The eggs used for the second series of experiments were approximately from 2 to 4 hours old when treated. For the injection series larvae were grown in culture dishes rich in yeast, and larvae just prior to pupation were used.

From table 1 it can be seen that there has resulted an increase in the lethal mutations rate of the *X*-chromosome from treatment with CuSO_4 in both series of experiments, injection of a 0.1% solution and treatment of naked, fertilized eggs with a concentrated aqueous solution for 10 minutes. The percentage of lethals obtained in each group was approximately the same. The ratio of the percentage difference to the standard error of the percentage difference for both groups combined is 2.5. The ratios reported by Magrzhikovskaja¹² for chorion-intact, fertilized eggs, treated with concentrated aqueous and alcoholic solution for longer time periods, were somewhat higher. See table 2.

The lethal mutation rate obtained in the manganese series is relatively higher than in the Ringer's injected controls, but not significantly so. A total of 3 lethals was obtained in 852 tested chromosomes, none in the injection series. In the concentrated aqueous KMnO_4 series reported by Naumenko¹³ there is likewise a greater percentage of lethals reported in the experimental group, but again not a significant increase in the rate. However, in his series treated with concentrated KMnO_4 in alcohol the lethal mutation rate is significantly greater than the control group. This holds true if a correction is made of his reported mutation rate 4.12 per cent. Of 24 lethals obtained, 18 appeared in 3 groups originating from 3 parents. The corrected rate should read 1.55 per cent (table 2).

Although Kondakowa and Popowa and Samjatina reported data suggesting that iodine treatment may produce mutations, the treatment resorted to in this study, injection of 0.038 per cent I_2 in 0.075 per cent KI , and immersion of naked eggs in a 50 per cent solution of I_2 in KI for 5 minutes did not produce a significant increase in the lethal mutation rate of the *X*-chromosome. Ssacharow,¹⁴ also, failed to induce mutations in

the X-chromosome through treatment of fertilized eggs with I_2 in KI, but did find a significant increase in the mutation rate of the third chromosome.

It is of particular interest that of the lethals obtained in the copper series using 11 treated males there appeared no genetically identical mutations. In the case of egg treatment it might be expected that an effect of the chemical should be produced on germ cells of a very early embryonic stage, and in the case of injections into mature larvae that some grouping of lethals might occur due to the effect of the chemical on some spermatogonial progenitor, providing, of course, that all affected cells survive. Magrzhikovskaja, likewise, reported no grouping of the 43 lethals he obtained in the copper series.

No lethal mutations were obtained in a total of 985 tested X-chromosomes coming from males receiving injections of 0.0013 per cent colchicine or through egg treatments with concentrated and 0.5 per cent aqueous solutions.

Summary.— $CuSO_4$ is found to influence the lethal mutation rate of the X-chromosome in *Drosophila melanogaster*, either by injection into mature larvae or treatment of naked, fertilized eggs with sub-lethal dosages.

Both $KMnO_4$ and I_2 in KI bring about a slight increase in the lethal mutation rate, but these are not statistically significant increases.

A comparative study is made with the results obtained by other workers using these same chemical substances.

Colchicine is found to be ineffective in altering the lethal mutation rate although it does reportedly influence the mitotic processes.

- ¹ Parker Fellow from Harvard University to Stanford University in 1937-38.
- ² Morgan, T. H., *Amer. Nat.*, **48** (1914).
- ³ Mann, M. C., *Jour. Exptl. Zool.*, **54** (1923).
- ⁴ Muller, H. J., these PROCEEDINGS, **14** (1928).
- ⁵ Muller, H. J., *Sci. Monthly*, **29** (1929).
- ⁶ Muller, H. J., *Amer. Nat.*, **64** (1930).
- ⁷ Stadler, L. J., *Science*, **68**, 168 (1928).
- ⁸ Medvedev, N., *C. R. Acad. Sci., U. R. S. S., Ser. A5*, 230 (1931).
- ⁹ Lobašov, M., and F. Smirnov, *Ibid.*, Ser. A2, 307 (1934).
- ¹⁰ Lobašov, M., *Trav. Soc. Natur. Leningrad*, **63**, 271 (1934).
- ¹¹ Goldat, S. J., and V. N. Beliaieva, *Biol. Žurn*, **4**, 384 (1935).
- ¹² Magrzhikovskaja, K. V., *Bull. Biol. Med. Exp.*, **1** (1936).
- ¹³ Naumenko, B., *Ibid.*, **1** (1936).
- ¹⁴ Ssacharow, W. W., *Genetica*, **18** (1936).
- ¹⁵ Samjatina, N. D., and O. T. Popowa, *B. Th.*, **3** (1936).
- ¹⁶ Kondakowa, A. A., *Biol. Žurn*, **4**, 721 (1935).
- ¹⁷ Lobašov, M., *Genetica*, **19**, 2001 (1937).
- ¹⁸ Stubbe, H., *Angewandte Chemie*, **50**, 241 (1937).
- ¹⁹ Method of Beadle, G. W., C. W. Clancy, and Boris Ephrussi, *Proc. Roy. Soc. Lon.*, Series B, No. 826, 122 (1937).
- ²⁰ See Nebel, B. R., and M. L. Ruttle, *Jour. Hered.*, **29**, 1, 3 (1938) for early literature.
- ²¹ Demerec, M., *Genetics*, **22**, 469 (1937).
- * The stock designated x-ple carries in the X-chromosome the mutant genes *sc*, *ec*, *cv*, *cl*⁶, *v*, *g*⁸, *f* in that order.

AN INTERNAL SECRETION AFFECTING VIABILITY IN CRUSTACEA

BY FRANK A. BROWN, JR.

DEPARTMENT OF ZOOLOGY, NORTHWESTERN UNIVERSITY

Communicated October 21, 1938

Aside from the work of Koller (1930) in which he found that an eyestalk hormone appeared to function in the control of calcium deposition in the exoskeleton of crustaceans, nothing other than the chromatophoric activities of these hormones has been suggested. During the course of earlier work upon the effects of removal of eyestalks from *Palaemonetes vulgaris* upon the state of pigment dispersion in the chromatophores, I frequently observed that the eyestalkless animals gradually lost their glassy transparency over the course of a few days and could be readily distinguished from the normal animal in this respect. Equally conspicuous was the lack of loss of transparency when the blinding operation consisted only of the removal of the ommatidial portion of the stalk leaving the major portion of the stalk tissue intact. The difference in effects of these two types of operations was correspondingly registered in a completely different type of chromatophore behavior. At the time, a possible extra-chromatophoric activity of the eyestalk hormones was not followed up.

With the discovery of glands comparable with the eyestalk glands of decapod crustaceans in lower crustaceans which are not equipped with functional pigment cells (Stahl 1938) and in the head regions of such other arthropods as the Insecta (Hanström 1935), it has suggested itself more and more strongly that the chromatophoric hormones of crustaceans, or substances closely related to them in place of formation, have other and fundamental functions in the general body metabolism. It is the purpose of this brief report to describe some initial experiments that have been performed upon crayfishes and which demonstrate quite conclusively the importance of an eyestalk hormone in the normal continuation of the life of the individual.

During the course of such experiments five species of crayfishes have been used with substantially similar results with each. The particular experiments reported here, however, are the most extensive ones and were conducted with the species *Cambarus blandingi acutus*. These animals ranged in carapace length from 12 to 30 mm.

The first experiment involved treating animals in four different ways. One lot of crayfishes had both eyestalks removed, another had the eyestalks removed but with the stalk tissue implanted into the ventral abdominal region, a third lot suffered removal of a single eye, while the fourth lot was entirely untreated. The animals were placed in individual finger bowls

in which the water was about $1\frac{1}{2}$ cm. deep. The bowls were loosely covered with glass plates.

A second experiment was performed to determine the exact extent to which the length of postoperative life could be increased by the transplantation procedure. For this experiment two lots of crayfishes were subjected to eyestalk removal; the first lot was given eyestalk tissue injected into the ventral abdominal region in Ringer's solution; the second lot had a similar quantity of clear Ringer's solution injected in a similar manner.

In a third experiment crayfishes after removal of the eyestalks were placed in a refrigerator at a temperature of about 7 degrees Centigrade.

In all the experiments the operative procedure was quite simple. The eyestalks were removed at their base with a sharp, pointed scalpel and the wound promptly seared over with an electric cautery needle. When the animal was to receive an abdominal implant of eyestalk tissue, the two stalks were immediately placed in a drop of Ringer's solution and the chitinous exoskeleton removed from them with fine forceps. Care was taken to remove all the living tissue with the exception of bits of the hypodermis. The larger pieces of tissue were then teased to pieces and the suspension injected by means of a glass pipette into the ventral abdomen through an aperture made in the fifth or sixth segment. The pipette tip was pushed anteriorly so that the introduced tissue came to occupy a position in the first, second and third segments and was clearly visible through the transparent cuticle. The total volume of the injected mass seldom exceeded 0.05 cc. When an eyestalkless animal was to serve as a control for the effect of the implant, an injection of Ringer's solution was made in the same way as has just been described. With the use of such a control the results demonstrated a more decisive difference between eyestalkless animals with and without the tissue implant than in the first experiment where the difference in the severity of the operations tended to draw together the averages of their lengths of life. The effect of the abdominal injection itself seems to decrease the average postoperative life by about one day.

Table 1 indicates the results of each of the above experiments, giving the frequency distribution of the deaths of the animals over a period of days. In all of the experiments those animals which died within the first twenty-four hours after the operation (about 20 per cent) were not included in the data. The great majority of these deaths were attributable to operative shock or excessive loss of blood resulting from unsuccessful cautery of the stubs. Further justification for the omission of these was found in the distribution of deaths over a period of days. Neglecting the deaths of the first day the times of the deaths displayed fairly normal distribution. Among other advantages such omission permitted more accurate determination of the significance among the means.

Several facts are immediately evident as a result of these experiments. The eyestalks are essential to the normal continuation of life of these crayfishes, those animals having them removed dying in less than half the time

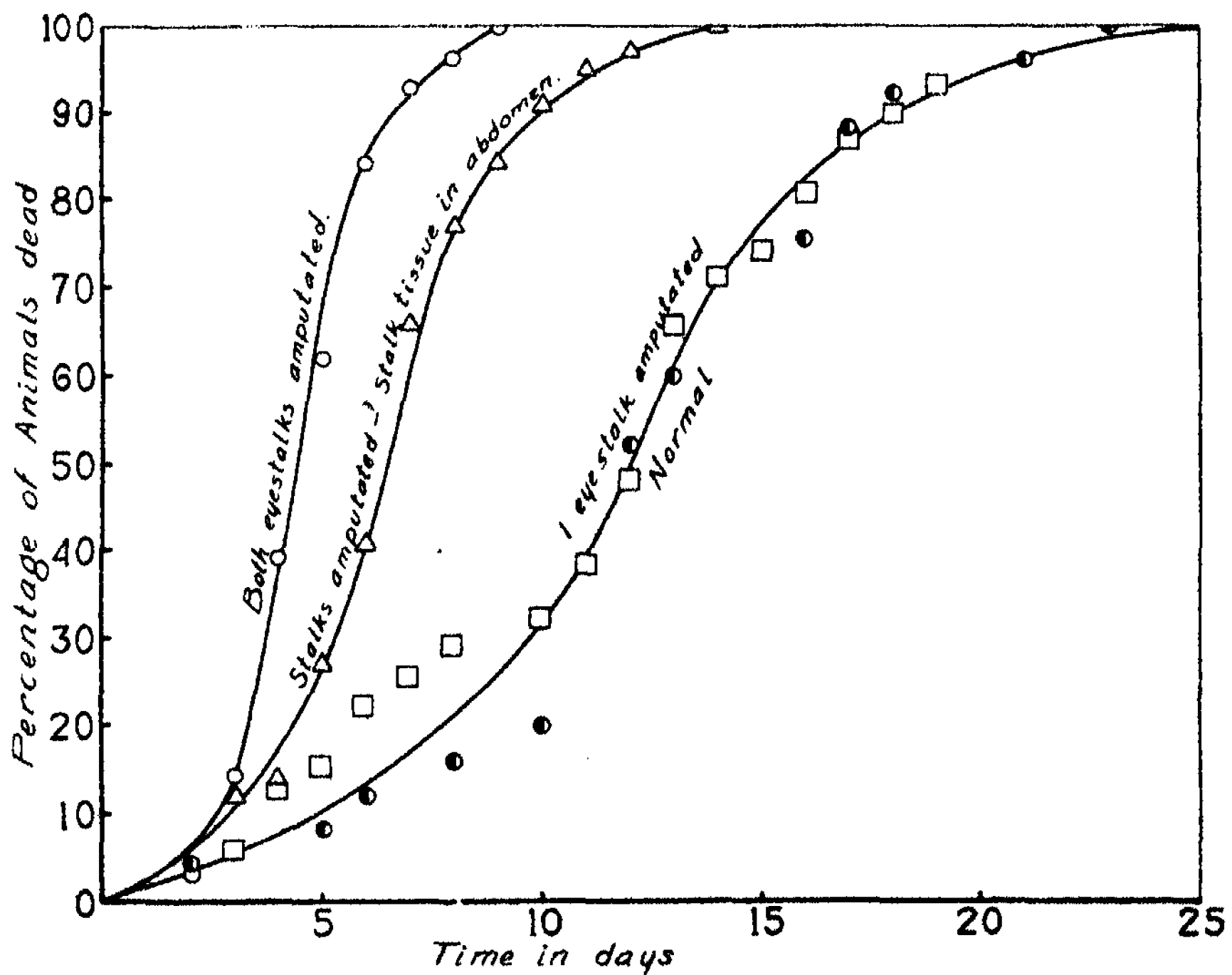
TABLE 1

DAYS	NORMAL	1 EYE OFF	EXP. I		EXP. II		EXP. III 2 EYES OFF 7°C.
			2 EYES OFF	2 EYES OFF INJ.	2 EYES OFF RINGER'S EYE TISS.	2 EYES OFF	
2	1	1	3
3	..	1	4	4	4	1	..
4	..	2	10	..	7	1	..
5	1	1	11	5	4	1	2
6	1	2	14	4	1	2	2
7	..	1	6	7	..	4	..
8	1	1	2	3	..	2	..
9	3	3	1
10	1	1	..	2	..	1	1
11	..	2	..	1
12	8	3	..	1	..	1	..
13	2	5
14	..	2	1	..
15	3	1
16	1	3
17	3	2	1
18	1	1
19	..	1
20	..	1
21	1
22
23	1
24	1	1
25
26	1
27
28	..	1
29
30
31	1
32
33
41	1
50	1
Av. Sur- vival	13.1	11.9	5.3	6.3	4.1	7.5	20.0
S. E.	±0.94	±0.98	±0.23	±0.42	±0.22	±0.81	

taken by normal ones. The definiteness of the necessity of the eyestalks for continued life is furthermore shown in the small standard error of the average length of postoperative life. On the other hand, it appears that a single remaining stalk is sufficient to maintain life over practically the

normal life span, there being no evidence in these results of a significant difference between the means of the normal animals and those from which a single eyestalk has been removed.

The implantation experiments lead strongly to the conclusion that the influence of the eyestalks upon viability is through a chemical control rather than because of any positional or structural relationship with the rest of the body. Even shredded bits of tissue in the abdominal region are capable of very significantly prolonging the life of eyestalkless animals. Thus far there has been no histological examination of the region of the implant to determine to what extent incorporation has gone on, or what particular



tissues of the stalks, if any, have persisted. When this is done it is possible that very interesting conclusions can be deduced as to the actual tissues involved in the activity. There is, however, some physiological evidence that there has been some incorporation of the elements involved in the production of the substance in question. This is seen in figure 1 which is a summary of experiments one and two combined. If the effects of the injection were one of a chemical substance introduced into the blood only at the time of the actual injection, then one would perhaps expect that there would be a more marked difference between the injected and the uninjected during the early postoperative period and then after the substance had

disappeared from the blood there would be equal rates of dying. That is not the case. The results indicate that some element is most effective in lowering the death rate after four or five days have elapsed since the injection. This finds ready explanation in the assumption of incorporation and subsequent activity of the implanted tissue.

The results thus far described pertain to experiments conducted at room temperature of about 25 degrees centigrade. Temperature has a very marked effect upon the length of time the animal is able to survive without eyestalks, as would be expected. Eyestalkless crayfishes kept at 7 degrees lived on the average of about 20 postoperative days as compared with the 5.3 days for ones kept at room temperature. The Q_{10} would thus be about 2.1.

It has perhaps already been noted that the normal animals lived in the conditions of the experiment only 12 days on the average. This shortness of life is undoubtedly the result of the absence of food coupled with a relatively high temperature and is assumed to be the time for starvation. None of the animals were fed during the course of the experiments. Being kept in separate containers, they were also unable to feed upon one another as they frequently do in the stock supply.

An attempt was made to determine whether there was any difference between sexes as to resistance to eyestalk removal. There appeared to be no difference whatsoever. Another attempt at correlation, that between size of the individual and resistance, appeared to indicate that through quite a range of sizes (carapace lengths from 12 to 30 mm.) there was none, but that animals somewhat larger than these dimensions were somewhat less affected by removal of the stalks. Too few of the larger animals have yet been used to establish this definitely.

It is much too early even to guess what relationship this vital substance bears to the chromatophore hormones of the eyestalk either with regard to chemical similarity or identity, or point of origin. However, there is definitely a chemical substance produced somewhere in the crayfish eyestalk essential to continued life of the animal.

Hanström, B., "Über eine Substanz im Insektenkopf, die zusammenballend auf das Pigment der Garneelenchromatophoren wirkt," *Kungl. Fys. Sällsk. Lund Forhandl.*, 6, No. 8 (1935).

Hanström, B., "Vermischte Beobachtungen über die Chromatophoraktivierenden Substanzen der Augenstiele der Crustaceen und des Kopfes der Insekten," *Kungl. Fys. Sällsk. Handl.*, 47, No. 8 (1937).

Koller, G., "Weitere Untersuchungen über Farbwechsel und Farbwechsellormone bei *Crangon vulgaris*," *Zeit. vergl. Physiol.*, 12 632-667 (1930).

Stahl, F., "Über das Vorkommen von inkretorischen organen und Farbwechsellormonen im Kopf einiger Crustaceen," *Kungl. Fys. Sällsk. Handl.*, 49, No. 12 (1938).

THE CONSISTENCY OF THE AXIOM OF CHOICE AND OF THE GENERALIZED CONTINUUM-HYPOTHESIS

BY KURT GÖDEL

THE INSTITUTE FOR ADVANCED STUDY

Communicated November 9, 1938

THEOREM. *Let T be the system of axioms for set-theory obtained from v. Neumann's system S^{*1} by leaving out the axiom of choice (Ax. III 3*); then, if T is consistent, it remains so, if the following propositions 1–4 are adjoined simultaneously as new axioms:*

1. The axiom of choice (i.e., v. Neumann's Ax. III 3*)
2. The generalized Continuum-Hypothesis (i.e., the statement that $2^{\aleph_\alpha} = \aleph_{\alpha+1}$ holds for any ordinal α)
3. The existence of linear non-measurable sets such that both they and their complements are one-to-one projections of two-dimensional complements of analytic sets (and which therefore are B_2 -sets in Lusin's terminology²)
4. The existence of linear complements of analytic sets, which are of the power of the continuum and contain no perfect subset.

A corresponding theorem holds, if T denotes the system of Princ. Math.³ or Fraenkel's system of axioms for set theory,⁴ leaving out in both cases the axiom of choice but including the axiom of infinity.

The proof of the above theorems is constructive in the sense that, if a contradiction were obtained in the enlarged system, a contradiction in T could actually be exhibited.

The method of proof consists in constructing on the basis of the axioms of T^5 a model for which the propositions 1–4 are true. This model, roughly speaking, consists of all "mathematically constructible" sets, where the term "constructible" is to be understood in the semiintuitionistic sense which excludes impredicative procedures. This means "constructible" sets are defined to be those sets which can be obtained by Russell's ramified hierarchy of types, if extended to include transfinite orders. The extension to transfinite orders has the consequence that the model satisfies the impredicative axioms of set theory, because an axiom of reducibility can be proved for sufficiently high orders. Furthermore the proposition "Every set is constructible" (which I abbreviate by " A ") can be proved to be consistent with the axioms of T , because A turns out to be true for the model consisting of the constructible sets. From A the propositions 1–4 can be deduced. In particular, proposition 2 follows from the fact that all constructible sets of integers are obtained already for orders $< \omega_1$, all constructible sets of sets of integers for orders $< \omega_2$ and so on.

The proposition A added as a new axiom seems to give a natural completion of the axioms of set theory, in so far as it determines the vague notion of an arbitrary infinite set in a definite way. In this connection it is important that the consistency-proof for A does not break down if stronger axioms of infinity (e.g., the existence of inaccessible numbers) are adjoined to T . Hence the consistency of A seems to be absolute in some sense, although it is not possible in the present state of affairs to give a precise meaning to this phrase.

¹ Cf. *J. reine angew. Math.*, **160**, p. 227.

² Cf. N. Lusin, *Leçons sur les ensembles analytiques*, Paris, 1930, p. 270.

³ Cf. A. Tarski, *Mh. Math. Phys.*, **40**, p. 97.

⁴ Cf. A. Fraenkel, *Math. Zeit.*, **22**, p. 250.

⁵ This means that the model is constructed by essentially transfinite methods and hence gives only a relative proof of consistency, requiring the consistency of T as a hypothesis.

THE CHARACTERIZATION OF PSEUDO- $S_{n,r}$ SETS

BY LEONARD M. BLUMENTHAL AND GEORGE R. THURMAN

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF MISSOURI

Communicated November 8, 1938

I. If to each pair of elements (*points*) p, q of an abstract set there is attached a non-negative real number (*distance*) pq , independent of the order of the elements, while $pq = 0$ if and only if $p = q$, the resulting space is called *semimetric*. A fundamental problem in the distance geometry of the n -dimensional spherical surface $S_{n,r}$ of radius r (the n -dimensional surface of a sphere of radius r in euclidean space of $n + 1$ dimensions, with "shorter arc" distance) consists in characterizing those semimetric spaces S which have the following properties: (1) S contains more than $n + 3$ points, (2) if $p, q \in S$, then $pq \neq d = \pi r$, (3) if p_1, p_2, \dots, p_{n+2} are elements of S , then there exists a function f mapping these $n + 2$ points upon $S_{n,r}$ with preservation of distances (i.e., *congruently*), (4) S cannot be mapped congruently upon a subset of $S_{n,r}$. Reserving the details of the investigation for publication elsewhere, we summarize in this note the complete solution of this problem. Semimetric spaces S with properties (3), (4) are called pseudo- $S_{n,r}$ sets.¹

The properties of the $S_{n,r}$, by virtue of which the characterization theorems of pseudo- $S_{n,r}$ sets are obtained, are all consequences of the following *metric* ones: (1) the mutual distances of each set of $n + 2$ points of $S_{n,r}$ satisfy a relation of the form $|\varphi(p_i p_j / r)| = 0$, ($i, j = 1, 2, \dots, n + 2$), where $\varphi(pq/r)$ is a real, single-valued, monotonically de-

creasing function defined over the *distance set* of $S_{n,r}$, with $\varphi(0) = 1$ and $\varphi(d/r) = -1$, $(\varphi(pq/r) \equiv \cos pq/r)$, (2) for each integer k , and each set of $k + 1$ points of $S_{n,r}$, the above determinant is non-negative, while there exists a set of $n + 1$ points for which the determinant is positive, (3) each set of $n + 1$ points with a non-vanishing determinant forms a *complete metric basis*, (4) if p_1, p_2, \dots, p_n, p are $n + 1$ points of $S_{n,r}$, with non-vanishing determinant, there exists at least one point p' , distinct from p , such that $pp_i = p'p_i$, ($i = 1, 2, \dots, n$).

II. *Characterization Theorems.*—The two following characterization theorems are obtained.

FIRST CHARACTERIZATION THEOREM. *If P is a pseudo- $S_{n,r}$ set containing more than $n + 3$ points and without diametral points (i.e., no pair of points of P has a distance equal to d), then every pair of distinct points of P has the distance $r \cdot \cos^{-1}(\pm 1/(n + 1))$. Not every distance equals $r \cdot \cos^{-1}(1/(n + 1))$.*

SECOND CHARACTERIZATION THEOREM. *Let P be a pseudo- $S_{n,r}$ set containing more than $n + 3$ points and without diametral points. Then for every positive integer k , the determinant $|\cos p_i p_j / r|$, ($i, j = 1, 2, \dots, k + 1$), formed for $k + 1$ points of P has (upon multiplication of appropriate rows and the same numbered columns by -1) ALL elements outside the principal diagonal equal to $-1/(n + 1)$.*

Thus, a pseudo- $S_{n,r}$ set containing more than $n + 3$ points, and without diametral points, is essentially equilateral, with distance $r \cdot \cos^{-1}(-1/(n + 1))$. It is, of course, obvious that a set of arbitrary power exceeding $n + 2$ and with every distance equal to $r \cdot \cos^{-1}(-1/(n + 1))$ is a pseudo- $S_{n,r}$ set without diametral points, but that every pseudo- $S_{n,r}$ set containing more than $n + 3$ points, and without diametral points, has essentially this simple structure is surprising. Many interesting conclusions follow from this result.

¹ For $n = 1$ the term pseudo- d -cyclic is used. The characterization of pseudo- d -cyclic sets has been given (Blumenthal, L. M., *Amer. Jour. Math.*, **54**, 387–396, 729–738 (1932); **56**, 225–232 (1934) but the treatment for higher dimensions demands quite different methods. The case $n = 2$ is solved in *Distance Geometries*, University of Missouri Studies, 1938. The general case involves several departures from the procedure adopted there.

ON SEMI-GROUPS OF SELFADJOINT TRANSFORMATIONS IN HILBERT SPACE

BY BÉLA DE SZ. NAGY

SZEGED, HUNGARY

Communicated November 5, 1938

In a paper dealing with the representations of Lie groups by linear transformations in the n -dimensional and Hilbert space,¹ I have obtained the following particular result:

Let H_t ($-\infty < t < \infty$) be a family of bounded selfadjoint transformations in Hilbert space \mathfrak{H} , forming a group, i.e., $H_0 = E$ (the identical transformation), $H_t H_s = H_{t+s}$. Suppose that (for every element f in \mathfrak{H}) the numerical function $(H_t f, f) = |H_{t/2} f|^2$ of t is Lebesgue-measurable in a suitable neighborhood of $t = 0$. Let us denote by m resp. M the lower resp. the upper bound of H_1 (we have $m > 0$). Then there is a resolution of the identity, E_λ , continuous from the left, such that $E_\lambda = 0$ for $\lambda \leq m$, $E_\lambda = E$ for $\lambda > M$ and that

$$H_t = \int_0^\infty \lambda^t dE_\lambda. \quad (1)$$

The same conclusion holds if we suppose boundedness, instead of measurability, of the function $(H_t f, f)$ in a neighborhood of $t = 0$.

Recently, E. Hille has considered the case of families H_t ($0 < t < \infty$) of positive definite selfadjoint transformations forming semi-groups, i.e., such that $H_s H_t = H_{s+t}$ for $s > 0$, $t > 0$.² Making the assumption that the upper bounds of the $H_t - E$ are less than, or equal to, 1, and using a theorem on the Laplace-Stieltjes representation of absolutely monotone functions, he gets a spectral representation for H_t essentially of the form of (1).

In this short note I shall show how a result on semi-groups, containing that of Hille's, can be obtained by the same method as used in my paper. This result reads as follows:

Let H_t ($0 < t < \infty$) be a family of founded selfadjoint transformations in \mathfrak{H} , forming a semi-group, i.e., $H_s H_t = H_{s+t}$ for $s > 0$, $t > 0$. Suppose that (for every element $f \in \mathfrak{H}$) the numerical function $(H_t f, f) = |H_{t/2} f|^2$ of t satisfies in a certain interval $a \leq t \leq b$ (a and b may depend upon f) at least one of the following conditions: 1) it is bounded, 2) it is measurable. Denote by m and M the lower and upper bounds of H_1 respectively (as $H_1 = (H_{1/2})^2$, we have $m \geq 0$). Then there is a resolution of the identity, E_λ , continuous from the left, such that $E_\lambda = 0$ for $\lambda \leq m$, $E_\lambda = E$ for $\lambda > M$ and

$$H_t = \int_0^\infty \lambda^t dE_\lambda.$$

Demonstration.—Using Schwarz's inequality, we see that the function $h_f(t) = \log (H_t f, f)$ (f arbitrary, but fixed) is a convex function of t on $0 < t < \infty$:

$$\begin{aligned} 2h_f((t+s)/2) &= \log (H_{(t+s)/2} f, f) = \log (H_{t/2} f, H_{s/2} f) \\ &\leq \log (|H_{t/2} f|^2 \cdot |H_{s/2} f|^2) = h_f(t) + h_f(s). \end{aligned}$$

We know further that $h_f(t)$ is bounded from above, or that it is measurable, in a certain interval $a \leq t \leq b$. But a convex function enjoying in a sub-interval of its domain one of these properties is necessarily continuous in the whole interior of its domain.³ $h_f(t)$ and therefore $(H_t f, f)$ are thus continuous for $0 < t < \infty$. As f was arbitrary, H_t must vary itself continuously with the parameter t .

Let now E_λ be the resolution of the identity corresponding to H_1 ; we have then $E_\lambda = 0$ for $\lambda \leq m$, $E_\lambda = E$ for $\lambda > M$ (m and M denoting the bounds of H_1 , $m \geq 0$). Putting

$$G_s = \int_0^\infty \lambda^s dE_\lambda \quad (s > 0),$$

we get a new semi-group of bounded selfadjoint transformations, $G_1 = H_1$. H_t being permutable with H_1 , is also permutable with G_s . Putting $(G_{1/2} - H_{1/2})f = g$, we have

$$\begin{aligned} |G_{1/4}g|^2 + |H_{1/4}g|^2 &= (G_{1/2}g, g) + (H_{1/2}g, g) = ((G_{1/2} + H_{1/2}) \\ &\quad (G_{1/2} - H_{1/2})f, g) = ((G_{1/2}^2 - H_{1/2}^2)f, g) = ((G_1 - H_1)f, g) = 0. \end{aligned}$$

Thus $G_{1/4}g = H_{1/4}g = 0$ and therefore also $G_{1/2}g = H_{1/2}g = 0$. It follows that $|(G_{1/2} - H_{1/2})f|^2 = ((G_{1/2} - H_{1/2})^2 f, f) = ((G_{1/2} - H_{1/2})g, f) = 0$, $G_{1/2} = H_{1/2}$. Repeating these arguments, we can verify the identity of H_t and G_t for $t = 1/2^n$ ($n = 0, 1, 2, \dots$). The semi-group property yields then this identity for $t = m/2^n$ ($m, n = 1, 2, 3, \dots$). Finally, as a result of the continuity, we get $H_t = G_t$ for all values of t .

¹ Béla v. Sz. Nagy, "Über messbare Darstellungen Liescher Gruppen," *Mathematische Annalen*, **112**, 286-296 (1936).

² Einar Hille, "On Semi-Groups of Transformations in Hilbert Space," *Proc. Nat. Acad. Sci.*, **24**, 159-161 (1938).

³ Cf. J. L. W. L. Jensen, "Sur les fonctions convexes etc.," *Acta Mathematica*, **30**, 175-193 (1906); W. Sierpiński, "Sur les fonctions convexes mesurables," *Fundamenta Mathematicae*, **1**, 125-129 (1920).

GROUPS OF ORDER p^m CONTAINING $m - \alpha$ INDEPENDENT GENERATORS

By G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated October 31, 1938

If a group of order p^m , p being a prime number, contains a set of $m - \alpha$ independent generators then every set of its independent generators involves exactly $m - \alpha$ operators, and the cross-cut of its subgroups of index p is invariant, of the order p^α and the corresponding quotient group is abelian and of type $1^{m-\alpha}$. We shall here confine our attention to the case when this cross-cut is cyclic. When the group G is abelian there is one and only one such group in view of the obvious theorem that every abelian group of order p^m is completely determined by m and the cross-cut of its subgroups of index p . This group in the present case is of type $\alpha + 1, 1^{m-\alpha-1}$ and hence its properties are well known. In what follows it will therefore be assumed that G is non-abelian unless the contrary is stated. It is obvious that G cannot involve any operator whose order exceeds $p^{\alpha+1}$ since the p th power of each of its operators appears in each of its subgroups of index p . When p is odd and $m > 2$ it is known that there is at least one group of order p^m which has the property that it involves no operator whose order exceeds p and that each of its subgroups of index p involves a given subgroup of order p . In this group $\alpha = 1$ but the group contains no operator of order $p^{\alpha+1}$.

It is easy to prove that in every other case G involves operators of order $p^{\alpha+1}$. In the special case when $p = 2$ it is well known that not all the operators of G besides the identity can be of order 2 since it is assumed that G is non-abelian. If G contains no operator of order $2^{\alpha+1}$ it contains a subgroup of order $2^{\alpha+2}$ which involves a commutator subgroup of order 2^α and hence contains an operator which transforms a commutator of order 2^α into its inverse. The given subgroup of order $2^{\alpha+2}$ therefore involves an abelian subgroup of order $2^{\alpha+1}$ which gives rise to a commutator of order 2^α under this subgroup of order $2^{\alpha+2}$. As this is impossible it has been proved that G involves operators of order $2^{\alpha+1}$ when $p = 2$. When $p > 2$ it results directly that it would involve a subgroup of index p which would not include the given cyclic subgroup of order p^α , $\alpha > 1$, if it did not contain an operator of order $p^{\alpha+1}$. Hence there results the following theorem: *If a group of order p^m has the property that the cross-cut of all its subgroups of index p is the cyclic group of order p^α then it has $m - \alpha$ independent generators and involves operators of order $p^{\alpha+1}$, whenever $\alpha > 1$, but no operator of a higher order. In the special case when $p = 2$, it is not necessary to assume that $\alpha > 1$.*

Since the case when $p = 2$ involves various considerations which do not present themselves when p is an odd prime number it will be assumed in what follows that p is odd unless the contrary is stated. The commutator subgroup of G is of order p since the p th transform of every operator of G with respect to the powers of a given operator is the operator itself. All the operators of order p contained in G , together with the identity, constitute an invariant subgroup of G and all of the operators of G whose orders divide a given power of p less than $p^{\alpha+1}$ constitute such a subgroup which is of index p under the group generated by the operators of G whose orders divide the next larger power of p . Since the cross-cut of all the subgroups of index p contained in G is generated by every operator of order $p^{\alpha+1}$ contained in G and these operators of order $p^{\alpha+1}$ generate G it results that this cross-cut appears in the central of G . It results from the dihedral group of order 2^m , $m > 3$, that this theorem does not in general apply to the case when $p = 2$. Some of the other theorems of this paragraph do also not apply to this special case which is excluded for the present.

Since G is assumed to be non-abelian it must include operators of order p which are non-invariant, for if all its operators of order p would be in its central then all its operators whose orders divide p^{α} would be invariant, but this is impossible since the central quotient group of every group is non-cyclic. It results from the quaternion group that this theorem is also not generally true when $p = 2$. Let s_1 be a non-invariant operator of order p contained in G and considered the subgroup of index p under G composed of all of its operators which are commutative with s_1 . If this subgroup involves an operator of order $p^{\alpha+1}$ then G involves an operator s_2 of order p which is non-commutative with s_1 and the two operators s_1, s_2 generate the non-abelian group of order p^3 which involves no operator of p^2 . If the subgroup of index p composed of all the operators of G which are commutative with s_1 does not involve any operator of order $p^{\alpha+1}$ then it is either the abelian group of type $\alpha, 1, m - \alpha - 1$ or it involves a non-invariant operator of order p .

In the former case all the operators of order $p^{\alpha+1}$ contained in G are non-invariant and G is completely determined, being constructed by adjoining to the given abelian group an operator of order $p^{\alpha+1}$ which transforms one of its independent generators of order p into itself multiplied by a commutator of order p . If this subgroup of index p contains no operator of order $p^{\alpha+1}$ and is non-abelian it contains two non-invariant operators s_3 and s_4 which together generate the non-abelian group of order p^3 which involves no operator of order p^2 since the given subgroup of index p contains invariant operators of order p^{α} . Hence two distinct cases present themselves. In one of these G contains invariant operators of order $p^{\alpha+1}$ while in the other it contains no invariant operator whose order exceeds p^{α} . It will be convenient to consider these two cases separately, beginning with

the former. The method of procedure will be to construct an infinite system of groups coming under each of these two cases and then to prove that such a system is composed of all the possible groups which come thereunder.

Starting with the abelian group of type $\alpha + 1, 1^\beta$ we extend this group by an operator t_1 of order p which is commutative with each of the operators in a reduced set of its independent generators except with one t_2 of those of order p . If $\beta > 1$ we may repeat this process any arbitrary number of times not exceeding β times. It is obvious that each of the groups thus obtained satisfies the conditions imposed on G and hence the total number of such distinct groups is $(m - \alpha - 1)/2$ when $m - \alpha$ is odd and exceeds unity and $(m - \alpha - 2)/2$ when $m - \alpha$ is even and exceeds 2. The smallest group in this category is the non-abelian group of order p^4 which involves a cyclic central of order p^2 but no operator of order p^3 . It is easy to see that every G whose central involves an operator of order $p^{\alpha+1}$ is a group contained in this system. In particular, when $m - \alpha$ is an odd positive number greater than unity there are $(m - \alpha - 1)/2$ groups of order p^m , p being an odd prime number, which separately have the properties that the cross-cut of all their subgroups of index p is the cyclic group of order p^α and that their centrals involve operators of order $p^{\alpha+1}$. When $m - \alpha$ is an even positive number there are $(m - \alpha - 2)/2$ such groups. In both cases each of these groups has $m - \alpha$ independent generators.

If we start with the abelian group of type $\alpha, 1^\beta$ we can similarly extend this group when $\beta > 0$ by an operator s_1 of order $p^{\alpha+1}$ which has its p th power in this abelian group and is commutative with each of the operators in a reduced set of its independent generators except with one s_2 of those of order p which it transforms into itself multiplied by one of its operators of order p which are generated by one of its operators of order $p^{\alpha+1}$. When $\beta > 1$ we can extend the group thus obtained by an operator N_3 of order p which is commutative with every operator in the given set of independent generators of the said abelian group of type $\alpha, 1^\beta$ except with one s_4 of order p which differs from s_2 and which it transforms into itself multiplied by the same commutator of order p as s_2 was transformed. The subgroup generated by s_3 and s_4 is the non-abelian group of order p^3 which involves no operator of order p^2 , and the subgroup generated by s_1 and s_2 is the non-abelian group of order $p^{\alpha+2}$ which involves operators of order $p^{\alpha+1}$. All the operators of one of these two subgroups are commutative with every operator of the other. When $\beta > 2$ this process may be repeated an arbitrary number of times not exceeding β times.

The smallest group which belongs to this category is the non-abelian group of order p^3 which involves operators of order p^2 . It is obvious that the direct product of an abelian group of type 1^k and any one of the groups noted above satisfies the conditions imposed on G and that when $m - \alpha$

is an even number the number of the distinct groups which come under the second of these categories is $(m - \alpha)/2$ and when $m - \alpha$ is an odd number the number of the distinct groups which come thereunder is $(m - \alpha - 1)/2$. Hence the groups of order p^m which have the property that the cross-cut of all their subgroups of index p is the cyclic group of order p^α present comparatively little difficulty when p is an odd prime number. In the special case when $\alpha = m - 1$ it is well known that there is only *one* such non-abelian group but when this cross-cut is of order p and $m > 3$ there are two categories of such groups composed respectively of those groups which have invariant operators of order p^2 and those which involve no such operators. In general there are two and only two groups for a given value of m and a given central whenever the order of the central quotient group is a given even power of p . When it is a given odd power of p there is no such group.

When $p = 2$ and the cross-cut of all the subgroups of index 2 contained in G is a cyclic group of order 2^α two cases present themselves. In one of these G is generated by its operators of order $2^{\alpha+1}$ and hence the given cyclic subgroup of order 2^α is in the central of G and the commutator subgroup of G is of order 2. This case is similar to the case considered above when p is odd and hence requires no special treatment here. It will therefore be assumed in what follows that the operators of order 2^α in the given cyclic subgroup of order 2^α are non-invariant under G and hence $\alpha > 1$. Since each of the cyclic subgroups of order $2^{\alpha+1}$ contained in G is invariant under G the given operators of order 2^α are transformed into their inverses under G and hence G contains a subgroup of index 2 in which the operators of the given cyclic subgroup of order 2^α are invariant. This subgroup is generated by the operators of order $2^{\alpha+1}$ contained in G and hence is either abelian or has a commutative subgroup of order 2.

In the former case it is of type $\alpha + 1$, $m - \alpha - 2$ and the remaining operators of G may transform all the operators of this subgroup into their inverses and thus give rise to two groups. In one of these all of the remaining operators are of order 2 while in the other all of these operators are of order 4. There is one additional group in which the remaining operators of G transform every operator of this abelian group into its $2^\alpha - 1$ power. In this case one-half of these additional operators are of order 2 while the rest are of order 4. In each of these cases the corresponding commutator subgroup is the cyclic group of order 2^α and each of the operators of order 2 in the given abelian subgroup of index 2 under G corresponds to itself. Another isomorphism of this group with respect to the cyclic group of order 2 can be established whenever the order of the given abelian group exceeds $2^{\alpha+1}$ by letting some of the operators of order 4 correspond to themselves. Hence there is another G which involves the given abelian group as a subgroup of index 2. In this group half of the remaining operators

are also of order 2 while the rest are of order 4. There are therefore four such G 's which involve this abelian subgroup of index 2.

It remains to consider the case when the given subgroup of index 2 has a commutator subgroup of order 2 but when the operators of order 2^α in the cross-cut of all the subgroups of index 2 under G are not invariant under G . Since each of the cyclic subgroups of order $2^{\alpha+1}$ contained in G is again invariant under G the given operators of order 2^α are transformed into their inverse by half of the operators of G . The remaining operators of G are therefore of orders 2 and 4, since when all the operators of a group which do not appear in a subgroup of index 2 are of order 2 this subgroup is necessarily abelian. It is therefore necessary to consider subgroups of the given subgroup of index 2 which give rise to a cyclic quotient group of order 2^α with respect to this subgroup. These subgroups can be selected in various ways depending upon whether the given subgroup of index 2 involves invariant operators of order $2^{\alpha+1}$ or does not have this property. The number of distinct cases to be considered is somewhat large but the separate cases present little difficulty.

INDEX

- ABRAMOWITZ, M. See Steinberg, A. G., 107.
- Affine geometry (Menger), 486.
- Algebras (Brauer), 243.
- Analytic functions (Walsh and Seidel), 337; (Walsh), 477.
- ANDERSON, R. L. See Beadle, G. W., 80.
- Anemone, Pacific Coast (Fox and Moe), 230.
- Approximation (Walsh), 477.
- Archaeology of Southeast Asia (deTerra), 407.
- Ascorbic acid (Bonner and Bonner), 70.
- Asia, Southeastern (deTerra), 407.
- Astacene-like carotenoid (Fox and Moe), 230.
- Asymmetry of light curves (Gaposchkin), 1.
- Autosomal linkage in man (Burks), 512.
- Auxin (van Overbeek), 42; (van Overbeek and Bonner), 260.
- Axiom of choice (Gödel), 556.
- BABCOCK, H. D. Some Recent Observations of Sunspot Spectra, 525.
- Bacterial populations (Gordon), 212.
- Bar eye of *Drosophila Melanogaster* (Chevais, Ephrussi and Steinberg), 365.
- Bar "locus" and the v^+ reaction (Steinberg and Abramowitz), 107.
- Barium stearate (Buchwald, Gallagher, Haskins, Thatcher and Zahl), 204.
- BATE, R. C. See Kaufmann, B. P., 368.
- BATEMAN, H. The Lift and Drag Functions for an Elastic Fluid in Two Dimensional Irrotational Flow, 246.
- . Rayleigh Waves, 315.
- . Coulomb's Function, 321.
- BEADLE, G. W., ANDERSON, R. L., AND MAXWELL, J. A Comparison of the Diffusible Substances Concerned with Eye Color Development in *Drosophila*, *Ephestia* and *Habrobracon*, 80.
- BERNSTEIN, F. The Continuumproblem, 101.
- Bioelectric potentials, calculations of (Hill and Osterhout), 312.
- BIRKHOFF, G. Dependent Probabilities and Spaces (L), 154.
- BLINKS, L. R., AND SKOW, R. K. The Time Course of Photosynthesis as Shown by the Glass Electrode, with Anomalies in the Acidity Changes, 413.
- . The Time Course of Photosynthesis as Shown by a Rapid Electrode Method for Oxygen, 420.
- BLUMENTHAL, L. M., AND THURMAN, G. R. The Characterization of Pseudo- S_n sets, 557.
- BOK, B. J., AND LINDSAY, E. M. Note on the Stellar Distribution in the Vicinity of a Southern Galactic Window, 4.
- BONNER, D. See Bonner, J., 70.
- BONNER, J., AND BONNER, D. Ascorbic Acid and the Growth of Plant Embryos, 70.
- BONNER, J., AND BUCHMAN, E. R. Syntheses Carried Out in Vivo by Isolated Pea Roots: I, 431.
- BONNER, J. See van Overbeek, J., 260.
- Boundary conditions (Calkin), 38.
- BRAUER, R. On Normal Division Algebras of Index 5, 243.
- BRINK, R. A., AND COOPER, D. C. Partial Self-Incompatibility in *Medicago Sativa*, 497.
- BROWN, F. A., JR. An Internal Secretion Affecting Viability in Crustacea, 551.
- BUCHMAN, E. R. See Bonner, J., 431.
- BUCHWALD, C. E., GALLAGHER, D. M., HASKINS, C. P., THATCHER, E. M., AND ZAHL, P. A. Measurements of Resistance and Capacity of Monofilms of Barium Stearate, 204.
- BURKS, B. S. On the Relative Contributions of Nature and Nurture to Average Group Differences in Intelligence, 276.
- . Autosomal Linkage in Man—The Recombination Ratio between Congenital Tooth Deficiency and Hair Color, 512.
- Calculus, differential (Michal), 340.
- Calculus of variations (Herzberger), 466.
- CALKIN, J. W. Abstract Self-Adjoint Boundary Conditions, 38.
- Capacity of Monofilms (Buchwald, Gallagher, Hoskins, Thatcher and Zahl), 204.
- CARLSON, J. G. Mitotic Behavior of Induced Chromosomal Fragments Lacking Spindle Attachments in the Neuroblasts of the Grasshopper, 500.
- Carotenoid, astacene-like (Fox and Moe), 230.
- Catfish, color changes of (Osborn), 121.
- Chemicals, effect of on lethal mutation rate (Law), 546.
- CHEVAIS, S., EPHRUSSI, B., AND STEINBERG, A. G. Facet Number and the v^+ Hormone in the Bar Eye of *Drosophila Melanogaster*, 365.
- Chick embryos (Willier and Rawles), 446.
- Chromatids (Kaufmann and Bate), 368.
- Chromosome numbers (Wipf and Cooper), 87.
- Chromosome phylogeny (McClung), 439.
- Chromosomes, polytene (Cooper), 452.
- DE CICCO, J. See Kasner, E., 34, 393.
- Clebsch-Gordan formula (Murnaghan), 182.

- Colchicine effects (Eigsti), 56.
 Colloids, soil (Jenny and Overstreet), 384.
 Color changes of the catfish (Osborn), 121.
 Color of meteorites (Watson), 532.
 Common vetch (Wipf and Cooper), 87.
 Conformal geometry (Kasner and de Cicco), 393.
 Continuum-hypothesis (Gödel), 556.
 Constants, dimensionless (Haas), 274.
 Continuumproblem (Bernstein), 101.
 COOPER, D. C. See Wipf, L., 87, Brink, R. A., 497.
 COOPER, K. W. Concerning the Origin of the Polytene Chromosomes of Diptera, 452.
 Corpus Allatum (Scharrer and Hadorn), 236.
 Coulomb's function (Bateman), 321.
 COURANT, R. The Existence of a Minimal Surface of Least Area Bounded by Prescribed Jordan Arcs and Prescribed Surfaces, 97.
 —. Remarks on Plateau's Problem and Douglas' Problem, 519.
 CROZIER, W. J., AND HOLWAY, A. H. On the Law for Minimal Discrimination of Intensities. III, 130.
 CROZIER, W. J., WOLF, E., AND ZERRAHN-WOLF, G. On the Duplexity of Visual Response in Vertebrates, 125.
 —. Temperature and the Critical Intensity for Response to Visual Flicker, 216.
 —. Specific Constants for Visual Excitation. II, 221.
 CROZIER, W. J., AND WOLF, E. On the Duplexity Theory of Visual Response in Vertebrates. II, 538.
 —. Specific Constants for Visual Excitation. III, 542.
 Crustacea (Brown), 551.
 Curve, Jordan space (Douglas), 490.
 Curves, transversal (Herzberger), 466.
 Cyclotomic fields (Vandiver), 330.
 Dependent probabilities (Birkhoff), 154.
 Derivatives of functions (Walsh and Seidel), 337.
 Differential calculus (Michal), 340.
 Diffusible substances concerned with eye color (Beadle, Anderson and Maxwell), 80.
 Dilution method (Gordon), 212.
 DILWORTH, R. P. See Ward, M., 162.
 Dimensionless constants of physics (Haas), 274.
 Diptera (Cooper), 452.
 Distribution of gene frequencies (Wright), 253.
 DOERING, C. R., AND FORBES, A. L. A Skeleton Life Table, 400.
 DOUDOROFF, P. See Sumner, F. B., 459, 463.
 DOUGLAS, J. Remarks on Riemann's Doctoral Dissertation, 297.
 —. Minimal Surfaces of Higher Topological Structure, 343.
 —. Green's Function and the Problem of Plateau, 353.
 —. The Most General Form of the Problem of Plateau, 360.
 —. A Jordan Space Curve Having the Infinite Area Property at Each of Its Points, 490.
 Drag functions (Bateman), 246.
 Drosophila (Beadle, Anderson and Maxwell), 80; (Kaufmann and Bate), 368.
 Drosophila Melanogaster (Steinberg and Abramowitz), 107; (Scharrer and Hadorn), 236; (Chevais, Ephrussi and Steinberg), 365; (Law), 546.
 Drosophila Virilis (Lebedeff), 165.
 Drosophila, X-rayed (Haskins and Enzmann), 136.
 Duplexity theory (Crozier, Wolf and Zerrahn-Wolf), 125; (Crozier and Wolf), 538.
 Duplication, intercalary (Kaufmann and Bate), 368.
 Early man (deTerra), 407.
 Eggs, fucus (Whitaker), 85.
 EIGSTI, O. J. A Cytological Study of Colchicine Effects in the Induction of Polyploidy in Plants, 56.
 Einstein spaces (Fialkow), 30.
 Elastic fluid (Bateman), 246.
 Element transformations (Kasner and de Cicco), 34.
 Embryo, chick (Willier, Rawles and Koch), 176; (Willier and Rawles), 446.
 Embryos, plant (Bonner and Bonner), 70.
 ENZMANN, E. V. See Haskins, C. P., 136.
 Eocene (Stock), 288.
 Ephesia (Beadle, Anderson and Maxwell), 80.
 EPHRUSSI, B. See Chevais, S., 365.
 Epiactis Prolifera (Fox and Moe), 230.
 Excitation, visual (Crozier and Wolf), 542.
 Expanding universe (Shapley), 148.
 Extensions of measure (MacNeille), 188.
 Extraction of auxin (van Overbeek), 42.
 Eye color development (Beadle, Anderson and Maxwell), 80.
 Facet number (Chevais, Ephrussi and Steinberg), 365.
 Feather characterization (Willier and Rawles), 446.
 FIALKOW, A. Einstein Spaces in a Space of Constant Curvature, 30.
 Fields, cyclotomic (Vandiver), 330.
 Fishes, disease in (Sumner and Doudoroff), 463.
 FLETCHER, H. The Mechanism of Hearing as Revealed Through Experiment

- on the Masking Effect of Thermal Noise, 265.
- Flicker, visual (Crozier, Wolf and Zerrahn-Wolf), 216.
- Flow, two dimensional irrotational (Bateman), 246.
- Fluid, elastic (Bateman), 246.
- Foraminifera, life cycle of (Myers), 10.
- FORBES, A. L. See Doering, C. R., 400.
- Forms, representation and equivalence of (Oldenburger), 193.
- FOSTER, A. L. Natural systems, 185.
- FOX, D. L., AND MOE, C. R. An Astaxanthin-Like Carotenoid from a Pacific Coast Anemone, *Epiactis Prolifera*, 230.
- FRED, E. B., WILSON, P. W., AND WYSS, O. Light Intensity and the Nitrogen Hunger Period in the Manchu Soybean, 46.
- Fucus eggs (Whitaker), 85.
- Function, Coulomb's (Bateman), 321.
- Function, Green's (Douglas), 353.
- Functional topology (Morse), 326.
- Functions, analytic (Walsh and Seidel), 337; (Walsh), 477.
- GALLAGHER, D. M. See Buchwald, C. E., 204.
- Galactic window, Southern (Bok and Lindsay), 4.
- Gambusia (Sumner and Doudoroff), 459.
- GAPOSCHKIN, S. Asymmetry of Light Curves in the Great Sequence as a Function of Frequency of Period, 1.
- Garden pea (Wipf and Cooper), 87.
- Gene frequencies (Wright), 253, 372.
- Generators, of groups (Miller), 17, 91, 94, 561.
- Geological discoveries (deTerra), 407.
- Geometry (Menger), 486.
- Geometry, conformal (Kasner and de Cicco), 393.
- GÖDEL, K. The Consistency of the Axiom of Choice and of the Generalized Continuum-Hypothesis, 556.
- GORDON, R. D. Note on Estimating Bacterial Populations by the Dilution Method, 212.
- Grasshopper, mitosis in (Carlson), 500.
- Great Sequence, light curves in (Gaposchkin), 1.
- Green's function (Douglas), 353.
- Group, real orthogonal (Murnaghan), 184.
- Groups (Richardson), 21.
- Groups (Miller), 17, 91, 94, 199, 202, 293, 333, 473, 561.
- Growth of plant embryos (Bonner and Bonner), 70.
- GUTH, E., AND HAAS, A. E. The Relations between Stefan's Radiation Law, Nernst's Heat Theorem and Maxwell's Formula for the Radiation Pressure, 224.
- HAAS, A. E. The Dimensionless Constants of Physics, 274.
- HAAS, A. H. See Guth, E., 224.
- Habrobracon (Beadle, Anderson and Maxwell), 80.
- HADORN, E. See Scharrer, B., 236.
- Halicystis, potentials in (Osterhout), 75.
- HASKINS, C. P., AND ENZMANN, E. V. A Determination of the Magnitude of the Cell "Sensitive Volume" Associated with the White-Eye Mutation on X-Rayed *Drosophila*. III, 136.
- HASKINS, C. P. See Buchwald, C. E., 204.
- Hearing, mechanism of (Fletcher), 265.
- HECHT, S., AND PICKELS, E. G. The Sedimentation Constant of Visual Purple, 172.
- HERZBERGER, M. Theory of Transversal Curves and the Connections between the Calculus of Variations and the Theory of Partial Differential Equations, 466.
- Hilbert space (Hille), 159; (de Sz. Nagy), 559.
- HILL, S. E., AND OSTERHOUT, W. J. V. Calculations of Bioelectric Potentials. III. Variation in Partition Coefficients and Ion Mobilities, 312.
- HILL, S. E. See Osterhout, W. J. V., 427.
- HILLE, E. On Semi-Groups of Transformations in Hilbert Space, 159.
- HOLWAY, A. H. See Crozier, W. J., 130.
- Homology groups (Richardson), 21.
- Hormones, melanophore-dispersing (Osborn), 121.
- Hormone, v^+ (Chevais, Ephrussi and Steinberg), 365.
- Hormones, synthetic male (Willier, Rawles and Koch), 176.
- Horn angles (Kasner and de Cicco), 393.
- Host-graft combinations (Willier and Rawles), 446.
- HUTCHINSON, G. E. Chemical Stratification and Lake Morphology, 63.
- Infectious disease in fishes (Sumner and Doudoroff), 463.
- Intelligence, Nature and nurture (Burks), 276.
- Intensities, minimal discrimination of (Crozier and Holway), 130.
- Intensity, critical (Crozier, Wolf and Zerrahn-Wolf), 216.
- Internal secretion (Brown), 551.
- Interpolation (Walsh), 477.
- Integers, singular (Vandiver), 330.
- Intersexuality in *Drosophila Virilis* (Lebedeff), 165.
- Ion mobilities (Hill and Osterhout), 312.
- JENNINGS, H. S. Sex Reaction Types and Their Interrelations in *Paramecium Bursaria*. I, 112.

- . Sex Reaction Types and Their Interrelations in *Paramecium Bursaria*. II. Clones Collected from Natural Habitats, 117.
- JENNY, H., AND OVERSTREET, R. Contact Effects between Plant Roots and Soil Colloids, 384.
- JONES, D. F. Translocation in Relation to Mosaic Formation in Maize, 208.
- Jordan space curve (Douglas), 490.
- KASNER, E., AND DE CICCIO, J. Classification of Element Transformations by Means of Isogonal and Equi-Tangential Series, 34.
- . Conformal Geometry of Horn Angles of Second Order, 393.
- KAUFMANN, B. P., AND BATE, R. C. An X-Ray Induced Intercalary Duplication in *Drosophila* Involving Union of Sister Chromatids, 368.
- KAVANAGH, F. See Robbins, W. J., 141, 145, 229.
- KOCH, F. C. See Willier, B. H., 176.
- Lake morphology (Hutchinson), 63.
- Lattices, residuated (Ward and Dilworth), 162.
- LAW, L. W. The Effects of Chemicals on the Lethal Mutation Rate in *Drosophila Melanogaster*, 546.
- LEBEDEFF, G. A. Intersexuality in *Drosophila Virilis* and Its Bearing on Sex Determination, 165.
- LEFSCHETZ, S. On Locally Connected Sets and Retracts, 392.
- LEWY, H. On the Existence of a Closed Convex Surface Realizing a Given Riemannian Metric, 104.
- Life cycle of the foraminifera (Myers), 10.
- Life table (Doering and Forbes), 400.
- Lift functions (Bateman), 246.
- Light curves, asymmetry of (Gaposchkin), 1.
- Light intensity and nitrogen hunger (Fred, Wilson and Wyss), 46.
- LINDSAY, E. M. See Bok, B. J., 4.
- Linear topological spaces (Michal), 340.
- Linkage, autosomal, in man (Burks), 512.
- Log π and other constants (Uhler), 23.
- McCLUNG, C. E. Chromosome Phylogeny, 439.
- MACNEILLE, H. M. Extensions of Measure, 188.
- Maize (Jones), 208.
- Maize, origin of (Mangelsdorf and Reeves), 303.
- Maize seeds (Maxwell), 377.
- Man, autosomal linkage in (Burks), 512.
- Man, early (deTerra), 407.
- MANGELSDORF, P. C., AND REEVES, R. G. The Origin of Maize, 303.
- MAXWELL, J. See Beadle, G. W., 80.
- MAXWELL, L. R. The Mechanism of Delayed Killing of Maize Seeds with X-Radiation, 377.
- Measure, extensions of (MacNeille), 188.
- Medicago Sativa* (Brink and Cooper), 497.
- Melanin content of *Gambusia* (Sumner and Doudoroff), 459.
- Melanophore-dispersing hormones (Osborn), 121.
- MENGER, K. A New Foundation of Non-Euclidean, Affine, Real Projective and Euclidean Geometry, 486.
- Metagalactic density gradient (Shapley), 282, 527.
- Meteorites (Watson), 532.
- Metric, Riemannian (Lewy), 104.
- MICHAL, A. D. Differential Calculus in Linear Topological Spaces, 340.
- MILLER, G. A. Groups Having a Maximum Number Set of Independent Generators, 17.
- . Groups Having a Maximum Set of Independent Generators of the Same Order, 91.
- . Groups Having a Maximum Number Set of Conjugate Independent Generators, 94.
- . Relative Numbers of Operators and Subgroups of a Finite Group, 199.
- . Minimum Degree of Substitutions of Highest Degree in a Group, 202.
- . Largest Degree of a Substitution in the Groups of a Given Degree, 293.
- . Groups of Degree n Involving only Substitutions of Lower Degrees, 333.
- . The Relative Numbers of the Subgroups and Operators of Certain Groups, 473.
- . Groups of Order p^m Containing $m - \alpha$ Independent Generators, 561.
- Minimal discrimination of intensities (Crozier and Holway), 130.
- Minimal surfaces (Courant), 97; (Douglas), 343.
- Mitotic behavior of induced chromosomal fragments (Carlson), 500.
- Mixodectid (Stock), 288.
- Mobilities, ion (Hill and Osterhout), 312.
- MOR, C. R. See Fox, D. L., 230.
- Monofilms (Buchwald, Gallagher, Haskins, Thatcher and Zahl), 204.
- Morphology, lake (Hutchinson), 63.
- MORSE, M. Functional Topology and Abstract Variational Theory, 326.
- Mosaic formation in maize (Jones), 208.
- MURNAGHAN, F. D. The Generalized Clebsch-Gordan Formula, 182.
- . The Analysis of Representations of the Real Orthogonal Group, 184.
- Mutation, irreversible (Wright), 253.
- Mutation rate, lethal (Law), 546.
- Mutation, white-eye (Haskins and Enzmann), 136.
- MYERS, E. H. The Present State of Our

- Knowledge Concerning the Life Cycle of the Foraminifera, 10.
- DE SZ. NAGY, B. On Semi-Groups of Self-adjoint Transformations in Hilbert Space, 559.
- Natural systems (Foster), 185.
- Nature and nurture (Burks), 276.
- Nernst's heat theorem (Guth and Haas), 224.
- Neuroblasts of grasshoppers (Carlson), 500.
- Nitella (Osterhout and Hill), 427.
- Nitrogen hunger of legumes (Fred, Wilson and Wyss), 46.
- Noise, thermal (Fletcher), 265.
- Non-Euclidean Geometry (Menger), 486.
- Normal division algebras (Brauer), 243.
- OLDENBURGER, R. Representation and Equivalence of Forms, 193.
- Operators (Miller), 199, 473.
- Origin of maize (Mangelsdorf and Reeves), 303.
- Orthogonal group (Murnaghan), 184.
- OSBORN, C. M. The Role of the Melanophore-Dispersing Hormone of the Pituitary in the Color Changes of the Catfish, 121.
- OSTERHOUT, W. J. V. Potentials in Halicystis as Affected by Non-Electrolytes, 75.
- OSTERHOUT, W. J. V., AND HILL, S. E. Reversal of the Potassium Effect in Nitella, 427.
- OSTERHOUT, W. J. V. See Hill, S. E., 312.
- VAN OVERBEEK, J. A Simplified Method for Auxin Extraction, 42.
- VAN OVERBEEK, J., AND BONNER, J. Auxin in Isolated Roots Growing *in Vitro*, 200.
- OVERSTREET, R. See Jenny, H., 384.
- Paramecium Bursaria (Jennings), 112, 117.
- Partial differential equations (Herzberger), 466.
- Partition coefficients (Hill and Osterhout), 312.
- Pea roots, syntheses by (Bonner and Buchman), 431.
- pH, effect of (Whitaker), 85.
- Photosynthesis, time course of (Blinks and Skow), 413, 420.
- Phylogeny, chromosome (McClung), 439.
- Physics, constants of (Haas), 274.
- PICKELS, E. G. See Hecht, S., 172.
- Pituitary (Osborn), 121.
- Phycomyces Blakesleeanus (Robbins and Kavanagh), 141, 145.
- Plateau's problem (Douglas), 353, 360; (Courant), 519.
- Polyploid populations (Wright), 372.
- Polyploidy in plants (Eigsti), 56.
- Polytene chromosomes (Cooper), 452.
- Populations, bacterial (Gordon), 212.
- Populations of polyploids (Wright), 372.
- Potassium effect in Nitella (Osterhout and Hill), 427.
- Potentials, bioelectric (Hill and Osterhout), 312.
- Potentials in halicystis (Osterhout), 75.
- Primate, Tarsiid (Stock), 288.
- Probabilities, dependent (Birkhoff), 154.
- Projective geometry (Menger), 486.
- Pseudo- $S_{n,r}$ sets (Blumenthal and Thurman), 557.
- Pyrimidine specificity of (Robbins and Kavanagh), 141.
- Radiation pressure (Guth and Haas), 224.
- RAWLES, M. E. See Willier, B. H., 176, 446.
- Rayleigh waves (Bateman), 315.
- Red clover (Wipf and Cooper), 87.
- REEVES, R. G. See Mangelsdorf, P. C., 303.
- Reflectivity of meteorites (Watson), 532.
- Representation and equivalence of forms (Oldenburger), 193.
- Residuated lattices (Ward and Dilworth), 162.
- Resistance of monofilms (Buchwald, Gallagher, Haskins, Thatcher and Zahl), 204.
- Retracts (Lefschetz), 392.
- RICHARDSON, M. Special Homology Groups, 21.
- Riemannian metric (Lewy), 104.
- Riemann's doctoral dissertation (Douglas), 297.
- Ring-gland, structure of (Scharrer and Hadorn), 236.
- ROBBINS, W. J. Organisms Requiring Vitamin B₁, 53.
- ROBBINS, W. J., AND KAVANAGH, F. The Specificity of Pyrimidine for Phycomyces Blakesleeanus, 141.
- . The Specificity of Thiazole for Phycomyces Blakesleeanus, 145.
- . Evidence for a Second Thiamin, 229.
- Roots, auxin in (van Overbeek and Bonner), 260.
- Roots, pea (Bonner and Buchman), 431.
- Roots, plant (Jenny and Overstreet), 384.
- SCHARRER, B., AND HADORN, E. The Structure of the Ring-Gland (Corpus Allatum) in Normal and Lethal Larvae of *Drosophila Melanogaster*, 236.
- Secretion, internal (Brown), 551.
- Sedimentation constant (Hecht and Pickels), 172.
- Seeds, maize (Maxwell), 377.
- SEIDEL, W. See Walsh, J. L., 337.
- Self-incompatibility in *Medicago Sativa* (Brink and Cooper), 497.

- Semi-groups (Hille), 159; (de Sz. Nagy), 559.
- Sespe (Stock), 507.
- Sets, locally connected (Lefschetz), 392.
- Sets, pseudo- $S_{n,r}$ (Blumenthal and Thurman), 557.
- Sex (Willier, Rawles and Koch), 176.
- Sex determination (Lebedeff), 165.
- Sex reaction types (Jennings), 112, 117.
- SHAPLEY, H. Note on the Problem of the Expanding Universe, 148.
- . A Metagalactic Density Gradient, 282.
- . Second Note on a Metagalactic Density Gradient, 527.
- Skow, R. K. See Blinks, L. R., 413, 420.
- Soil colloids (Jenny and Overstreet), 384.
- Soybean, Manchou (Fred, Wilson and Wyss), 46.
- Space, Hilbert (Hille), 159; (de Sz. Nagy), 559.
- Spaces, Einstein (Fialkow), 30.
- Spaces (L) (Birkhoff), 154.
- Spaces, linear topological (Michael), 340.
- Spectra, sunspot (Babcock), 525.
- Stefan's law (Guth and Haas), 224.
- STEINBERG, A. G., AND ABRAMOWITZ, M. The Bar "Locus" and the v^+ Reaction in *Drosophila Melanogaster*, 107.
- STEINBERG, A. G. See Chevais, S., 365.
- Stellar distribution (Bok and Lindsay), 4.
- Stock, C. A Tarsiid Primate and a Mixodectid from the Poway Eocene, California, 288.
- . A Titanotherium from the Type Sespe of California, 507.
- Stratification, chemical (Hutchinson), 63.
- Substitutions (Miller), 202, 293, 333.
- SUMNER, F. B., AND DOUDOROFF, P. Some Effects of Light Intensity and Shade of Background upon the Melanin Content of *Gambusia*, 459.
- . The Effects of Light and Dark Backgrounds upon the Incidence of a Seemingly Infectious Disease in Fishes, 463.
- Sunspot spectra (Babcock), 525.
- Surfaces, closed convex (Lewy), 104.
- Surfaces, minimal (Courant), 97; (Douglas), 343.
- Syntheses by pea roots (Bonner and Buchman), 431.
- Synthetic male hormones (Willier, Rawles and Koch), 176.
- Systems, natural (Foster), 185.
- Tarsiid primate (Stock), 288.
- DE TERRA, H. Preliminary Report on Recent Geological and Archaeological Discoveries Relating to Early Man in Southeast Asia, 407.
- THATCHER, E. M. See Buchwald, C. E., 204.
- Thermal noise (Fletcher), 265.
- Thiamin (Robbins and Kavanagh), 229.
- Thiazole, specificity of (Robbins and Kavanagh), 145.
- THURMAN, G. R. See Blumenthal, L. M., 557.
- Time course of photosynthesis (Blinks and Skow), 413, 420.
- Titanotherium (Stock), 507.
- Tooth deficiency and hair color (Burks), 512.
- Topological spaces, linear (Michael), 340.
- Topology, functional (Morse), 326.
- Transformations, element (Kasner and de Cicco), 34.
- Transformations in Hilbert space (Hille), 159.
- Transformations, selfadjoint (de Sz. Nagy), 559.
- Translocation (Jones), 208.
- Transversal curves (Herzberger), 466.
- UHLER, H. S. Log π and Other Basic Constants, 23.
- Universe, expanding (Shapley), 148.
- VANDIVER, H. S. On Criteria Concerning Singular Integers in Cyclotomic Fields, 330.
- Variational theory (Morse), 326.
- Variations, calculus of (Herzberger), 466.
- Vertebrates, visual responses in (Crozier, Wolf and Zerrahn-Wolf), 125; Crozier and Wolf, 538.
- Visual excitation (Crozier, Wolf and Zerrahn-Wolf), 221; (Crozier and Wolf), 542.
- Visual purple (Hecht and Pickels), 172.
- Visual response (Crozier, Wolf and Zerrahn-Wolf), 125; (Crozier and Wolf), 542.
- Vitamin B₁ (Robbins), 53.
- WALSH, J. L., AND SEIDEL, W. On the Derivatives of Functions Analytic in the Unit Circle, 337.
- WALSH, J. L. On Interpolation and Approximation by Functions Analytic and Bounded in a Given Region, 477.
- WARD, M., AND DILWORTH, R. P. Residuated Lattices, 162.
- WATSON, F. G. Reflectivity and Color of Meteorites, 532.
- Waves, Rayleigh (Bateman), 315.
- WHITAKER, D. M. The Effect of pH on the Development of Ultra-Centrifuged *Fucus* Eggs, 85.
- WILLIER, B. H., AND RAWLES, M. E. Feather Characterization as Studied in Host-Graft Combinations between Chick Embryos of Different Breeds, 446.
- WILLIER, B. H., RAWLES, M. E., AND KOCH, F. C. Biological Differences in the Action of Synthetic Male

- Hormones on the Differentiation of Sex in the Chick Embryo, 176.
- WILSON, P. W. See Fred, E. B., 46.
- WIPF, L., AND COOPER, D. C. Chromosome Numbers in Nodules and Roots of Red Clover, Common Vetch and Garden Pea, 87.
- WOLF, E. See Crozier, W. J., 125, 216, 221, 538, 542.
- WRIGHT, S. The Distribution of Gene Frequencies under Irreversible Mutation, 253.
- . The Distribution of Gene Frequencies in Populations of Polyploids, 372.
- WYSS, O. See Fred, E. B., 46.
- X-Rayed *Drosophila* (Haskins and Enzmann), 136.
- ZAHL, P. A. See Buchwald, C. E., 204.
- ZERRAHN-WOLF, G. See Crozier, W. J., 125, 216, 221.

I. A. R. I. 75

IMPERIAL AGRICULTURAL RESEARCH
INSTITUTE LIBRARY
NEW DELHI.

Date of issue.	Date of issue.	Date of issue.
13.12.52		
17.1.52		
15.7.52		
1 FEB 1955		
5.9.1963		
22.7.64		
24.4.67		
7-8-67		
16 NOV 1968		
4 JUN 1969		
3-5-91		